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(54) Title: A NOVEL TUMOR MARKER AND NOVEL METHOD OF ISOLATING SAME

(57) Abstract

The invention encompasses a novel tumor marker which is present on tumor cells and absent on correponding normal cells, nucleic acid encoding the tumor marker, and a novel method of isolating DNA encoding the tumor marker or a gene which is differentially expressed in tissues.

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A NOVEL TUMOR MARKER AND NOVEL METHOD OF ISOLATING SAME

FIELD OF THE INVENTION

This invention relates to proteins that serve as tumor markers for human carcinoma and to methods of isolating differentially expressed genes.

GOVE..MENT RIGHTS

This invention was made in part with U.S. Government support. Therefore, the U.S. Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Tumor markers for human tumor cells have been largely limited to activated oncogenes and their products, for example, the myc, ras, fos, and erbB2 genes and their encoded oncoproteins. In addition, activated anti-oncogenes, such as RB, p53, and DCC, have been identified in normal cells but do not appear to be present in tumor cells. Oncogene and anti-oncogene products have proven difficult to use as consistent predictors of tumor and normal tissue, respectively, due to the relatively low level of expression of the genes encoding these proteins. Thus, there is a need in the art for a tumor marker which is not only differentially expressed in tumor and normal tissue, but also consistently detectable in human tumor tissue and consistently absent in the corresponding normal tissue.

A common method used to identify genes differentially or uniquely expressed in tumors, in cells responding to growth factors, and in differentiated cell types such as, among others, T cells, adipocytes, neurons, and hepatocytes is the subtractive hybridization technique (S.W. Lee et al., Proc. Natl. Acad. Sci. USA 80:4699, 1983). A method of differential display of eukaryotic mRNA by means of the polymerase chain reaction (PCR) has recently been developed (P. Liang et al., Science 257:967, 1992). This method

utilizes oligo dT linked to two additional bases as the primer for reverse transcription driven by reverse transcriptase. cDNA fragments are then amplified by Taq DNA polymerase-based PCR using an oligo dT primer along with one additional primer. The amplified cDNAs are then resolved by DNA sequencing gels. There is a need in the art for a streamlined and simplified process for isolating cDNAs corresponding to differentially expressed mRNAs.

SUMMARY OF THE INVENTION

The invention is based on the discovery of a novel protein, TC1 (SEQ ID NO:4), which is a tumor marker, particularly for invasive and metastatic tumors, and the gene encoding this protein.

The invention thus encompasses the TC1 protein (SEQ ID NO:4), which is useful as a tumor marker for tumor diagnosis and therapy, particularly for colorectal, breast, and gastrointestinal tumors, and for metastatic tumors emanating from these tumor types. TC1 is also a useful marker in general for tumor cell invasion and metastasis. mRNA encoding TC1 is not expressed in most cultured tumor cells, i.e., *in vitro*, but is expressed once these cells are grown *in vivo*. Because later stage and deeply invasive tumors contain higher levels of TC1 protein than other tumor tissues, TC1 appears to be a particularly useful marker for later stage cancers.

TC1 protein may also serve as a target in tumor targeted therapy to prevent tumor cell metastasis and thus invasion of additional organs. For example, a polypeptide fragment of the TC1 protein may be used as an antagonist of TC1 biological activity; e.g., where TC1 biological activity includes invasion and metastasis, the polypeptide fragment may be administered to a patient afflicted with the tumor in order to inhibit the spread of the tumor to other tissues. Alternatively, a truncated portion of TC1 which retains the invasive and metastatic biological activities of the full-

length molecule will be useful for screening for antagonists of TC1 activity. Potentially useful polypeptides are described herein.

The invention also encompasses nucleotide probes based on the TC1 nucleotide sequence; e.g., 10, 20, 30, 40, etc. nucleotides in length. Such probes are useful for PCR-based tumor detection and *in situ* hybridization of tumor tissue sections. In addition, probes whose nucleotide sequences are based on homologies with other genes or proteins having sequences related to TC1, i.e., genes of the TC1 family, two of which are described herein, are useful for detecting additional genes belonging to the TC1 family of genes.

The invention thus also encompasses methods of screening for agents which inhibit expression of the TC1 gene (SEQ ID NO:3)
in vitro, comprising exposing a metastatic cell line in which TC1 mRNA is detectable in cultured cells to an agent suspected of inhibiting production of the TC1 mRNA; and determining the level of TC1 mRNA in the exposed cell line, wherein a decrease in the level of TC1 mRNA after exposure of the cell line to the agent is indicative of inhibition of TC1 mRNA production.

Alternatively, the screening method may include *in vitro* screening of a metastatic cell line in which TC1 protein is detectable in cultured cells to an agent suspected of inhibiting production of the TC1 protein; and determining the level of TC1 protein in the cell line, wherein a decrease in the level of TC1 protein after exposure of the cell line to the agent is indicative of inhibition of TC1 protein production.

The invention also encompasses *in vivo* methods of screening for agents which inhibit expression of the TC1 gene, comprising
exposing a mammal having tumor cells in which TC1 mRNA or protein is detectable to an agent suspected of inhibiting production of TC1 mRNA or protein; and determining the level

of TC1 mRNA or protein in tumor cells of the exposed mammal. A decrease in the level of TC1 mRNA or protein after exposure of the mammal to the agent is indicative of inhibition of TC1 gene expression.

These screening methods are particularly applicable to breast tumor cells, colon tumor cells, or tumor cells of the gastrointestinal tract.

The invention also encompasses a pharmaceutical composition for use in treating a late stage cancer, comprising an effective amount of an inhibitor of TC1, and a method of treating late stage cancer, comprising administering to a mammal afflicted with a late stage cancer a therapeutically effective amount of an inhibitor of TC1. Late stage cancers include those which have become deeply invasive in a tissue or which have metastasized to other tissues.

TC1 is detectable in patient blood, urine, sputum or other body fluid using a monoclonal antibody specific for a TC1 epitope. Thus, the invention also encompasses antibodies specific for TC1, which can easily be prepared in a kit form. Monoclonal antibodies specific for TC1 may be used for tumor imaging to localize tumor position and size. TC1-specific monoclonal antibodies are also useful as screening and diagnostic agents in immunohistochemical staining of tissue sections to distinguish tumor cells from normal cells. Thus, anti-TC1 antibodies are particularly useful where they recognize cells which produce the TC1 protein when such cells are paraffin-embedded and/or formalin-fixed. One example of such an antibody is the monoclonal antibody anti-TC1-1 produced by the hybridoma deposited with the American Type Culture Collection as ATCC Deposit No. HB 11481.

In another aspect, the invention also features a novel method, called palindromic PCR, for identifying and isolating a gene, e.g., a gene which is differentially expressed in different types of tissues. The method is based on the use

of short DNA primers and corresponding palindromic nucleotide sequences in the nucleotide sequence to be isolated.

Thus, the invention encompasses a method for producing a double stranded cDNA that includes the steps of contacting an mRNA with a DNA primer under stringent hybridization conditions to form a first hybrid molecule, the primer having a length of from 8 to 12 nucleotides and, preferably, 9 to 11 nucleotides; subjecting the first hybrid molecule to an enzyme having reverse transcriptase activity, to produce a first DNA strand complementary to at least a portion of the mRNA; contacting the first DNA strand with the primer under stringent hybridization conditions to form a second hybrid molecule; and subjecting the second hybrid molecule to an enzyme having DNA polymerase activity, to produce a second DNA strand complementary to the first DNA strand. Preferably, the method also includes the step of amplifying the first and second DNA strands.

In preferred embodiments, a single enzyme provides both the reverse transcriptase activity and the DNA polymerase activity. One example of a suitable such enzyme is rTth DNA polymerase from the thermophilic eubacterium *Thermus thermophilus*.

As used herein, the term "palindromic nucleotide sequences" means that a double stranded DNA molecule contains a specific DNA sequence in both its coding strand and its anti-parallel strand, when those strands are read in the same direction, e.g., 5' to 3'.

The specific sequence of the DNA primer is arbitrary in that it is based upon individual judgment. In some instances, the sequence can be entirely random or partly random for one or more bases. Preferably, the GC content of the primer is between 40% and 60%, most preferably about 50%. In other instances, the arbitrary sequence can be selected to contain a specific ratio of each deoxynucleotide. The arbitrary sequence can also be selected to contain, or not

to contain, a recognition site for a specific restriction endonuclease.

The DNA primer can contain a sequence that is known to be a "consensus sequence" of an mRNA of known sequence. As defined herein, a "consensus sequence" is a sequence that has been found in a gene family of proteins having a similar function or similar properties. The use of a primer that includes a consensus sequence may result in the cloning of additional members of a desired gene family.

Palindromic PCR enables genes that are altered in their frequency of expression, as well as those that are constitutively or differentially expressed, to be identified by simple visual inspection and isolated. The method also allows the cloning and sequencing of selected mRNAs, so that the investigator may determine the relative desirability of the gene product prior to screening a comprehensive cDNA library for the full length gene product.

Further objects and advantages of the invention will be apparent in light of the following description and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a polyacrylamide gel of size-separated cDNAs that were reverse transcribed from paired mRNAs from colon carcinoma (T) and adjacent normal colon tissue (N) and subsequently amplified.

Fig. 2A is a gel in which the TC1 cDNA fragment identified in Fig. 1 was recovered and re-amplified.

Fig. 2B is a Northern Blot of three pairs of RNA from colon carcinoma (T) and their adjacent normal colon tissue (N) probed with 32P-labeled TC1 cDNA.

Fig. 3 shows the nucleotide sequence (described herein as SEQ ID NO:1) and corresponding amino acid sequence (SEQ ID NO:2) of the 636 bp partial TC1 clone.

Fig. 4 shows the nucleotide sequence (SEQ ID NO:3) and corresponding amino acid sequence (SEQ ID NO:4) of the full-length TC1 gene and protein.

Fig. 5 is a sequence comparison of the four internal homologous domains of TC1 (SEQ ID NO:4), each approximately 135 amino acids.

Fig. 6A is a schematic representation of the four repeats of TC1.

Fig. 6B is a proposed schematic arrangement of the four repeated domains and the N- and C-terminal domains.

Fig. 7 shows the amino acid sequence identity between TC1 (SEQ ID NO:4) and Big-h3 (SEQ ID NO:17).

Fig. 8 is a Northern Blot of five pairs of RNA from colon carcinoma (T) and adjacent normal colon tissue (N) probed with 32P-labeled Big-h3 cDNA, the bottom panel representing control RNA probed with 32P-labeled B-actin.

Fig. 9 shows amino acid sequence homology between TC1 (SEQ ID NO:4) and Fasciclin I from Grasshopper (GrF) (SEQ ID NO:18) and Drosophila (DrF) (SEQ ID NO:19).

Fig. 10 is a Schematic representation showing that, on average, in every 202 bases of sequence in one strand of cDNA, there is one 9-base sequence exactly palindromic to that in a region of its antiparallel strand.

Fig. 11 shows the relationship between the palindromic frequency and the number of bases in a putative DNA primer, as determined by cDNA Matrix analysis.

Fig. 12 is a schematic representation of the method of the invention, palindromic PCR, driven by the enzyme rTth DNA polymerase with one DNA primer in one reaction tube; the dotted line indicates mRNA and the solid line indicates cDNA; the short jagged line represents the single DNA primer.

Fig. 13A shows the effect of the length of the DNA primer on the cDNA amplification patterns; the length and nucleotide sequence of each primer are: A, 8-mer (5'-TGTCGAGA); B', 9-mer (5'-TGTCCAGAC); C', 10-mer (5'-

TGTCCAGATG) (SEQ ID NO:5); D', 11-mer (5'-TGTCCAGATGC) (SEQ ID NO:6); E', 12-mer (5'-TGTCCAGATGAC) (SEQ ID NO:7).

Fig. 13B shows the effect of the GC content of the DNA primer on the cDNA amplification patterns; the GC content and nucleotide sequence of each primer (10-mer) are: A', 40% (5'-TGTCCAGATA) (SEQ ID NO:8); B', 50% (5'-TGTCCAGATG) (SEQ ID NO:5); C', 60% (5'-TGTCCAGACG) (SEQ ID NO:9): D', 70% (5'-TGTCCAGCCG) (SEQ ID NO:10); E', 80% (5'-TGTCCCGCCG) (SEQ ID NO:11); F', 90% (5'-TGCCCGGCCG) (SEQ ID NO:12).

Fig. 13C shows the effect of the sequence specificity of the DNA primer on the cDNA amplification patterns; 10-mer primers with the same GC content but different sequences are: A', 5'-TGATGCACTC (SEQ ID NO:13); B', 5'-TGAGCTACTC (SEQ ID NO:14); C', 5'-TGACTGACTC (SEQ ID NO:15).

Fig. 13D shows palindromic PCR performed by rTth DNA polymerase (A) with reverse transcription cycles (RT cycles) and (B) without RT cycles.

Fig. 14 shows the identification of differentially expressed genes in human colon carcinoma.

Fig. 15 shows reamplification of the TC1 cDNA fragment isolated from colon carcinoma; the PCR product was analyzed on a 1.0% agarose gel; a 0.63 Kb cDNA fragment (arrow) was detected.

Fig. 16 is an autoradiogram of DNA sequencing gels showing the presence of PP1 primer sequence (5'-CTGATCCATG) (SEQ ID NO:16) at the 5'-end of both strands of the TC1 cDNA fragment; cloning sites are indicated by arrows, sequences below arrows are pBS (KS) vector sequences reading from T3 primer and T7 primer.

Fig. 17 is a Northern Blot of 24 pairs of colon carcinoma (T) and their adjacent normal tissue (N) probed with 32P-labeled TC1 cDNA.

Fig. 18 shows the Tumor/Normal RNA Ratio from Northern Blot results of Fig. 17.

Fig. 19 is a Northern Blot of RNA from carcinoma cells which result from metastasis from colon carcinoma to liver

(LM) and their adjacent normal liver (NL) probed with ^{32}P-labeled TC1 cDNA.

Fig. 20 shows a Northern Blot of RNA from breast cancer cell line MCF-7 (1) and colon cancer cell line CX-1 (2) cultured *in vitro*, and MCF-7 tumor (3) and CX-1 tumor (4) grown *in vivo* in nude mice.

Fig. 21 shows staining of formalin-fixed and paraffin-embedded colon tumor tissue sections using the monoclonal antibody anti-TC1-1 and avidin-biotin-peroxidase detection.

Fig. 22 shows staining as described in Fig. 21, except that panels A, C, D represent breast invasive ductal carcinoma and panel B, normal breast tissue.

Fig. 23 shows staining as in Fig. 13, except that panels A and B represent gastric carcinoma, and panels C and D, deeply invasive colon carcinoma.

Fig. 24 is a Western Blot analysis of protein samples from two pairs of colon carcinoma and their adjacent normal colon (A) and two pairs of breast carcinoma and their adjacent normal breast (B), using a monoclonal antibody against TC1 protein as a probe.

Fig. 25A shows the ethidium bromide staining pattern of an RNA gel in which the same amount of RNA from JMN (1) and JMN1B (2) cells is loaded per lane.

Fig. 25B is a Northern Blot analysis of RNA from malignant mesothelioma cells JMN1B (2) and JMN (1) using TC1 cDNA as a probe.

Fig. 26 is a Western blot using a monoclonal antibody against TC1 to probe JMN1B cells grown in conditioned medium and whole cell lysate.

Fig. 27 shows JMN1B cells fixed with paraformaldehyde without subsequent permeabilization in panels A and B, and JMN1B cells fixed with paraformaldehyde and then permeabilized in panels C and D.

Figs. 28A-28D show the corrected nucleotide sequence and corresponding amino acid sequence of the full length TC1 gene and protein.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

TC1 (SEQ ID NO:4) is a novel protein that is found in invasive and metastatic tumor cells. The nucleotide sequence (SEQ ID NO:3) encoding TC1 was found using a novel technique described herein as palindromic PCR, a technique which enables identification and cloning of a gene that is differentially expressed in tissues. Cloning and sequencing of the gene encoding TC1 and characterization of the protein is described below, along with examples of how the protein is detected in invasive and metastatic cancers. Examples describing additional uses of the TC1 protein and its fragments, the nucleotide sequence encoding TC1 and fragments thereof, and antibodies specific for TC1 are also included.

Identification, Cloning and Detection of Expression of the TC1 Gene

The identification, cloning, and differential detection of expression of the TC1 gene (SEQ ID NO:3) was performed as follows. A 636 bp cDNA fragment (SEQ ID NO:1) containing TC1 sequences was identified and isolated by a rapid method termed palindromic PCR, described herein, from human surgical colon carcinoma tissue. Briefly, paired mRNAs were isolated from colon carcinoma tissue and adjacent normal colon tissue from the same patient, then matched mRNAs were reverse transcribed to cDNA and subsequently amplified by the palindromic PCR method described herein, which utilizes one DNA primer. Both reverse transcription and PCR reactions were driven by a single enzyme, rTth DNA polymerase, in a single tube. ^{35}S or ^{33}P-labeled PCR cDNA fragments were resolved on a DNA sequencing gel. As shown in Fig. 1, paired mRNAs from colon carcinoma (T) and adjacent normal colon tissue (N) were reverse transcribed to cDNA and subsequently amplified by palindromic PCR. ^{35}S-labeled PCR cDNA fragments were then resolved on a DNA sequencing gel. A differential cDNA band (TC1) appeared to be present only in the tumor sample. This TC1 cDNA fragment was recovered from the

sequencing gel and then reamplified with the same palindromic primer. This 636 bp fragment is identified with a horizontal arrow in Fig. 2A.

TC1 gene expression was examined in colon carcinoma cells and in the corresponding adjacent colon tissue, and the results were as follows. Fig. 2B is a Northern Blot of three pairs of RNA from colon carcinoma (T) and their adjacent normal colon tissue (N) probed with ^{32}P-labeled TC1 cDNA. TC1 mRNA was over-expressed in all three cases of colon carcinoma, whereas only very weak TC1 message appeared in the adjacent normal tissue. In the bottom panel of the blot, control RNA was blotted with ^{32}P-labeled cDNA encoding B-actin.

Northern Blot analysis of several pairs of Tumor/Normal total RNA using a ^{32}P-labeled TC1 cDNA probe revealed that the TC1 mRNA size is about 3.6Kb. This first TC1 cDNA fragment was cloned into a pBluescript plasmid DNA vector strategies. Nucleotide sequence analysis revealed that this fragment contained 636bp with nucleotide sequences corresponding to the primer sequence at both 5′-ends of the double-stranded DNA (Fig. 3 and SEQ ID NO: 1). The corresponding predicted amino acid sequence is shown in Fig. 3 and provided in SEQ ID NO: 2. A search of the GenBank database with this cDNA fragment revealed that TC1 is a novel gene.

Nucleotide sequence analysis of the 636bp TC1 cDNA fragment obtained by the described differential display method revealed that it contained a partial open reading frame. Therefore, this 636bp cDNA fragment was used as probe to screen a cDNA library. Several overlapping clones were obtained and contained a 2997bp sequence. To obtain the complete open reading frame for TC1, a modified 5′-end RACE technique was used to amplify the TC1 coding regions. The nucleotide and deduced amino acid sequence of full-length TC1 is shown in Fig. 4 and provided in SEQ ID NOS: 3 and 4. The N-terminal signal sequence is underlined; one predicted N-linked glycosylation site (NDT) is boxed and a

polyadenylation signal (AATAAA) is indicated. The cDNA contains 3126bp with a potential polyadenylation sequences (AATAAA) at the 3'-end, beginning at residue 2963. The open reading frame (ORF) encodes a 777-amino acid protein with a calculated molecular weight of 86kD. The TC1 protein contains an amino-terminal signal peptide or secretory leader signal (ALPARILALALALAL), and one predicted site of N-linked glycosylation at amino acid residue 605 (NDT). One Cemokine B family motif (C-C) was found at amino acid residue 85 (C-C) of TC1.

Analysis of the deduced amino acid sequence (SEQ ID NO:4) revealed that TC1 contained four internal homologous domains of approximately 135 amino acids. A comparison of these repeats is shown in Fig. 5. Each boxed amino acid is identical with at least one other residue at that same position. The interdomain homologies range from 32% (between domains 2 and 4) to 18% (between domains 1 and 3). Some amino acid sequence such as TLF $\frac{A}{V}$P $\frac{T}{S}$NEAF, NGVIHXID are highly conserved between all four repeats. The notations $\frac{A}{V}$ and $\frac{T}{S}$ are used herein to indicate that alanine or valine, and threonine or serine, respectively, may be found at these positions. In addition, the notation X is used herein to indicate that this position may include any amino acid. Each repeat starts with the most divergent sequence. The four repeats occur between residues 139-537 and are uninterrupted by non-homologous domains. A schematic representation of the four repeats of TC1 is shown in Fig. 6A. The four homologous repeats suggest a tetrameric structure (Mclachlan 1980; Zinn et al, 1988) with two binding sites, one at each intrachain dimer. The four repeats of TC1 may serve as ligand binding sites, with the N-terminal or C-terminal domains serving as the functional domain. One possible arrangement of the four repeated domains and the N- and C-terminal domains is shown schematically in Fig. 6B.

The nucleotide and corresponding amino acid sequence of the TC1 gene and protein with a corrected leader signal sequence are given in Figs. 28A-28D.

Palindromic PCR

Described below is a novel technique used to identify the TC1 mRNA and prepare TC1 cDNA. Although the sequence of bases in a coding and antisense strand of a cDNA molecule are, in a sense, "mirror images" of one another, we have found that with surprising frequency a short sequence of bases, e.g. 9 or 10, in one strand will be found to have an exact copy in its anti-parallel strand. We call these sequences "palindromic" sequences. This phenomenon has been used to develop a method of cDNA isolation and amplification.

In order to determine the frequency of occurrence or "palindromic frequency" of these anti-parallel repeats, a computer program called DNA Matrix (DNA Strider 1.2) was used to analyze double stranded cDNAs which were randomly selected from the GenBank database. DNA matrix analysis revealed the palindromic frequency of double strand cDNA to be surprisingly high and led to our development of a relationship between the number of bases in the chosen sequence, the "palindromic bases," and the palindromic frequency. Single strand cDNA (the mRNA strand) and its anti-parallel strand were compared, each from the 5′ to 3′ end by the DNA Matrix program. For example, as illustrated in Fig. 10, on the average, in every 202 bases of sequence in one strand of cDNA, there is one 9-base sequence that is exactly duplicated to that in another region of its antiparallel strand. The palindromic frequency found in native cDNA is much higher than that which would be calculated from random composition, suggesting that the nucleotide composition of double-stranded cDNA follows certain palindromic rules. As shown in Fig. 11, the palindromic frequency dramatically decreases when the number of bases in the searched segment increases. The key numbers

of bases which lead to dramatic change of palindromic frequency are 9, 10 and 11 bases. This, then, is the theoretical basis for designing a primer for use in the DNA isolation and amplification method of the invention, palindromic PCR.

Table 1 presents the statistical data showing the palindromic frequency related to the number of bases in the searched segment.

Table 1

Palindromic Frequency Related to No. of Bases in Searched Segment as Revealed by cDNA Matrix Analysis

No. Bases in Searched Segment (X Bases)	Average Length to Find One X-Base Palindromic Sequence	Palindromic Frequency
7 bases	18 bases	0.4
9 bases	202 bases	0.048
11 bases	872 bases	0.015
13 bases	>1996 bases	<0.007

The principle of the method of palindromic PCR is shown in schematic representation in Fig. 12. The general strategy is to use a single primer and one enzyme combining both reverse transcriptase and DNA polymerase activities, e.g., rTth DNA polymerase (from the thermophilic eubacterium *Thermus thermophilus*), to perform both reverse transcription and polymerase chain reaction in one reaction tube. rTth DNA polymerase possesses a very efficient reverse transcriptase activity in the presence of $MnCl_2$ and a thermostable DNA polymerase activity in the presence of $MgCl_2$. The rTth DNA polymerase has been observed to be greater than 100-fold more efficient in coupled reverse transcription and PCR than the analogous DNA polymerase, Taq (T. W. Myers et al., Biochemistry <u>30</u>:7661, 1991). In this reaction, an appropriate primer would allow anchored annealing to some regions of certain mRNA species that contain sequence complementary to the palindromic primer. This subpopulation

of mRNAs is likely to be reverse transcribed by rTth DNA polymerase. A "Palindromic" primer apparently has a greater probability of anchoring to the coding regions of mRNA than oligodT primer. Once mRNAs are reverse transcribed to form a first strand cDNA species, the same primer can anneal to some regions of the first strand cDNA and function as the "Downstream primer" in a PCR reaction. The same primer can also function as the "Upstream primer." When the primer anchors to first strand cDNAs, the annealing position to various cDNA molecules should, in principle, be at different distances in different molecules from the first annealing position. Therefore, the amplified cDNA fragments from various mRNAs will be of different sizes. Once these PCR-generated cDNA fragments are labeled with ^{35}S-dATP or ^{33}P-dATP, they can be resolved as a ladder by DNA sequencing gels. A display of cDNAs originating from various mRNAs can then be visualized after autoradiography.

The selection of the specific palindromic primer depends on three important factors: the length, the GC content, and the sequence specificity. DNA Matrix analysis has indicated that the ideal length of a primer for an appropriate palindromic frequency is from 9 to 11 bases. Therefore, a set of primers from 8 base to 12 base in length with 50% GC content was chosen for study. Our results showed that 9, 10, and 11 base primers gave an appropriate number of cDNA fragments readily resolvable by DNA sequencing gels (Fig. 13). To identify the GC content of the primer most suitable for this method, a set of 10-mer primers with GC content ranging from 40% to 90% was tested. The results suggested that a GC content from 40% to 80% is acceptable (Fig. 13). However, primers with 40% to 60% GC content appear to yield better results. To examine the effect of the specific sequence of the primer, 10-mer primers of different sequences each having 50% GC content was tested. As predicted, different primers gave rise to different cDNA

patterns (Fig. 13). As little a difference as three bases led to totally different cDNA profiles.

cDNA patterns generated by palindromic PCR are highly stable. When the same conditions were used but the experiments repeated at different times, the patterns of the amplified cDNA fragments were highly reproduced, indicating the reliability of this method.

In order to be sure of detecting mRNAs with a low copy number, it was necessary to determine the sensitivity of this method. It has been reported that the amplification driven by rTth DNA polymerase is at least 100-fold greater than that by Taq polymerase. rTth DNA polymerase allows the detection of IL-1a mRNA, which has a very low copy number, in 80pg of total cellular RNA (T.W. Myers et al., Biochemistry 30:7661, 1991). Thus, the higher efficiency of rTth DNA polymerase ensures that the palindromic PCR method of the invention provides high sensitivity. In addition, because rTth polymerase is thermostable, it can also be used to perform several RT cycles (reverse transcription cycles), which means several copies of first strand cDNA can be obtained from a single copy of mRNA. The sensitivity of the method is increased by performing multiple RT cycles using rTth polymerase (Fig. 13).

The method of the invention was tested in a search for differences in mRNA expression between human colon carcinoma and the adjacent normal epithelium from a surgical specimen. Paired mRNA preparations were reverse transcribed with a palindromic primer 5'-CTGATCCATG (designated as PP-1 primer) (SEQ ID NO:16) in the presence of $MnCl_2$ followed by PCR with the same primer in the presence of $MgCl_2$ using rTth DNA polymerase. The reaction products were then analyzed by DNA sequencing gels. About 70-110 amplified cDNA fragments ranging from 100-700 bases from both preparations were detected (Fig. 14). Whereas overall cDNA patterns between tumor and normal tissue are similar, significant differences were detected by this method. Most cDNA bands showed the

same intensity between tumor and normal preparations, but two cDNA bands designated as TC1 and TC2 appeared with increased intensities in tumor tissue (Fig. 14). A sample reaction protocol is described below.

To 1.1 μl of double distilled (dd) H_2O is added 0.5 μl of 10X rTth DNA polymerase reverse transcriptase (RT) buffer (100mM Tris-Hcl, pH 8.3, 900mM KCl), 0.5 μl of 10mM $MnCl_2$, 0.4 μl of 2.50mM dNTP, 1.0 μl (0.50 μg) of one palindromic primer (9-11 mer), and 1.0 μl (100 ng) of mRNA to form Mix A in a total vol. of 4.5 μl. Mix A is heated in a 0.5 ml PCR tube at 65°C for 6 min and then at 37°C for 8 min. Next, 0.5 μl (1.25 unit) of rTth DNA polymerase is added, the reaction mixture is mixed well, spun briefly, incubated at 70°C for 12 min and then placed on ice. Mix B which consisting of 12.5 μl of dd H_2O, 2.0 μl of 10X chelating buffer (50% glycerol (v/v), 100mM Tris-HCl, pH 8.3, 1M KCl, 0.5% Tween 20), 2.0 μl of 25 mM $MgCl_2$ solution, 2.50 mM dNTP and 2.0 μl of ^{35}S-dATP (or ^{33}P-dATP) is dispensed in the amount of 20 μl into each 5.0 μl RT reaction mixture. The samples are mixed and spun briefly and then overlaid with 25 μl of mineral oil. The polymerase chain reaction is then started: 94°C for 40 sec., 40°C for 2 min., 72°C for 35 sec. (for 40 cycles, hold at 72°C for 4 min.), and then 4°C.

For cDNA analysis, 7 μl of a PCR sample is mixed with 4 μl of sequencing loading buffer, samples are incubated at 80°C for 3 min., and then placed on ice. 4.5 μl of the sample is loaded on a 6%-8% agarose DNA sequencing gel.

A gel slice containing a desirable cDNA band (such as TC1) was soaked in 200 μl of ddH_2O for 20 min and then separated from 3M paper with a clean forcep or a plastic pipette tip. The gel was removed and pounded with an autoclaved plastic pipette tip. Elution buffer (20 μl) was added and the mixture was vortexed and left at room temperature for 4 hrs or overnight. After centrifugation, cDNA fragments in 10 μl eluent were reamplified by rTth DNA polymerase with the same palindromic primer, as described.

After one 40-cycle PCR, the reamplified cDNA could be detected by agrose gels stained with ethidium bromide. The amount of cDNA generated was sufficient for cloning and preparing a probe for Northern Blot analysis. Fig. 15 shows the gel obtained when the TC1 cDNA band was subjected to elution and reamplification. Total PCR product of the TC1 fragment was 2.5 μg.

The reamplified TC1 cDNA fragment was treated with T4 DNA polymerase and cloned into pBluescript plasmid DNA vector at SmaI site by blunt end ligation. The nucleotide sequence of the TC1 fragment (SEQ ID NO:1) showed that a sequence identical to the PP1 primer (SEQ ID NO:16) is indeed present at the 5′-end of both strands of the TC1 fragment (Fig. 16). This result confirms that the 5′-ends of both complementary chains of the TC1 cDNA fragment used the same palindromic primer during palindromic PCR as discussed above. It also implies that the same palindromic primer sequence is present at the 5′-ends of both strands for every PCR product in the same reaction. These results establish that a single 9-11 base palindromic primer can effectively prime reverse transcription and then serve as both a "Downstream primer" and an "Upstream primer" in palindromic PCR amplification.

The method of the invention differs from other methods in a number of ways. In palindromic PCR, only a single primer (9-11 bases) is used and is sufficient to prime reverse transcription as well as to support subsequent PCR for a display of nearly 100 cDNA species. Because the pattern of amplified cDNAs depends on the sequence of the single palindromic primer, the species of mRNAs that are subjected to amplification can readily be controlled by a proper sequence of the palindromic primer. If a group or family of genes shares certain sequences, a primer can be chosen from such a sequence, and a specific display of this set of mRNAs can readily be performed. Likewise, computer analysis of the Genebank database may reveal additional sequences useful as a primer shared by a set of related

genes. The use of such a primer by the method of the invention would allow the display for the expression of a given set of genes. Palindromic PCR provides an easy, sensitive and economical way to identify and isolate differentially expressed genes related to tumor and other disease.

<u>Differential expression of TC1 DNA in normal tissue and tumor cells.</u>

Northern Blot analysis, as described above, confirmed the differential expression of TC1 mRNA in colon carcinoma tissue, and the absence of TC1 mRNA in the corresponding normal tissue. Evaluation of the expression of TC1 mRNA in additional cases of colon carcinoma at different stages was also undertaken. Surgical specimens of 24 cases of human primary colon carcinoma and 6 cases of liver metastases were examined by Northern hybridization of total RNA with ^{32}P-labeled TC1 probe.

A Northern Blot of 24 pairs of colon carcinoma (T) and their adjacent normal tissue (N) probed with ^{32}P-labeled TC1 cDNA is shown in Fig. 17. It is evident from the results that the level of TC1 mRNA in tumor tissue is much greater than the level in adjacent normal tissue in all 24 cases. The TC1 mRNA levels vary in different cases of carcinoma. Panels I and II show A: TC1 mRNA and B: Control; Panel III shows TC1 mRNA and control (Actin) mRNA.

Fig. 18 shows the Tumor/Normal RNA Ratio from Northern Blot results of Fig. 17. The horizontal line indicates the mean Tumor/Normal ratio. TC1 mRNA was abundantly expressed in all 24 cases of primary colon carcinoma and 6 cases of liver metastases, whereas only a small amount of TC1 mRNA was detected in a few cases of paired adjacent normal tissue. The mRNA level of TC1 was much greater in primary colon carcinoma than in paired adjacent normal colonic epithelium in all 24 cases. The Tumor/Normal ratio varied from 5.6 to 92, and the mean Tumor/Normal ratio being 32. The Tumor/Normal ratio, when plotted against the Duke's stage of

disease, gave evidence for increasing TC1 expression with increasing stage of colon carcinoma.

In all six cases of paired colon carcinoma metastatic to liver, the TC1 mRNA level was much higher in metastatic tumor than in adjacent normal liver tissue. Fig. 19 shows a Northern Blot of RNA from metastatic colon carcinoma to liver (LM) and their adjacent normal liver (NL) probed with ^{32}P-labeled TC1 cDNA. TC1 mRNA was expressed only in metastatic tumor in 5 of 6 samples. Only one sample of normal liver tissue expressed a very weak TC1 message. The Tumor/Normal ratio is greater than 64. These results suggested that differential expression of TC1 may be associated with human colorectal cancer progression and biological aggressiveness of the disease.

<u>*In vivo* and *in vitro* expression of TC1 mRNA</u>

The expression of TC1 mRNA in cultured cancer cells and *in vivo* tumor cells was analyzed and is described below. TC1 was overexpressed in tumor tissue *in vivo*. The expression of TC1 mRNA in cultured cancer cell lines *in vitro* was examined by Northern Blot analysis. RNAs isolated from twelve colon cancer cell lines (HT29, Clone A, MIP101, CX-1, Morser, CCL227, CCL228, etc.) derived from different stage of human colon carcinoma, two melanoma cell lines (LOX, A2058), one breast cancer cell line (MCF-7), two cervical cancer cell lines (Hela, A431), three bladder cancer cell lines (EJ, T24, MB49), one pancreas cancer cell line (CRL1420), two hepatoma cell lines (HepG2, HepG3) and four normal cell lines (FS-2, MRC-5, 498A, CV-1) were screened by Northern Blot analysis. However, the TC1 transcript could not be detected in all of these cell lines. This result suggested that TC1 expression was dramatically decreased or indeed turned off in cultured cancer cells. However, after cultured cancer cells were injected into nude mice to grow tumor *in vivo*, TC1 mRNA expression turned on again and its mRNA level could be detected by Northern Blot analysis.

Fig. 20 shows a Northern Blot of RNA from breast cancer cell line MCF-7 (1) and colon cancer cell line CX-1 (2) cultured *in vitro*, and MCF-7 tumor (3) and CX-1 tumor (4) grown *in vivo* in nude mice. TC1 mRNA in colon cancer cell line CX-1 and breast cancer cell line MCF-7 cultured *in vitro* could not be detected by Northern Blot analysis. After cultured CX-1 and HT29 cells were injected into nude mice to form tumors *in vivo*, TC1 mRNA was detectable by Northern Blot analysis, the TC1 mRNA levels being dramatically increased *in vivo*. This result suggests that TC1 gene expression was turned on or dramatically increased in the tumor cells *in vivo*. Thus, the differential expression of the TC1 gene appears to be related to invasion and metastasis of tumor cells *in vivo*. The regulation of TC1 gene expression *in vivo* and *in vitro* could be a very important model to understand tumorigenesis and tumor malignant behavior.

<u>Expression of TC1 protein</u>

The expression of TC1 protein in *in vivo* tumor cells, cultured carcinoma cells, and in corresponding normal cells was examined, and is described below. The TC1 gene (SEQ ID NO:3) was cloned into a plasmid expression vector, and recombinant TC1 protein (SEQ ID NO:4) was expressed in bacteria. Several monoclonal antibodies against the bacterially-produced TC1 protein were raised, as will be described below. A variety of formalin-fixed and paraffin-embedded tumor tissue sections were examined by immunohistochemical staining with a mouse monoclonal anti-TC1 antibody anti-TC1-1 using an avidin-biotinylated-peroxidase detection technique. Strong positive staining of TC1 was found in primary colon carcinoma (Fig. 21, panel A), colon carcinoma metastatic to liver (Fig. 21, panel C) and lymph node (Fig. 21, panel D), breast carcinoma (Fig. 22, panels A,C,D) and gastric carcinoma (Fig. 23, panels A,B). The TC1 protein level in tumor tissue is much greater than the level of TC1 in adjacent normal tissue (Figs. 21B, 23B).

These results, which represent the staining of sixteen cases of different stages of primary colon carcinoma, eight cases of colon tumor metastatic to liver and lymph node, fourteen cases of breast carcinoma and five cases of gastric carcinoma, suggested the following three conclusions. First, the TC1 protein level appeared to be different in different types of carcinoma, with protein levels being highest in breast carcinoma. Second, the advance edge of the deeper invasive tumor appeared stain stronger for TC1, suggesting a greater prevalence of TC1 protein at the advance edge of the tissue. Third, the move advanced stages of tumor appeared to contain more TC1 protein.

Fig. 24 is a Western Blot analysis of protein samples from two pairs of colon carcinoma and their adjacent normal colon (A) and two pairs of breast carcinoma and their adjacent normal breast (B), using a monoclonal antibody against TC1 protein as a probe. A major 86kd protein (arrow) was detected by anti-TC1 antibody in tumor samples (T) but not in normal samples (N). The Western Blot analysis confirmed that tumor tissue contained significantly more TC1 protein than the corresponding adjacent normal tissue.

TC1 gene expression

The presence of TC1 mRNA and protein in malignant mesothelioma cells was examined, and is described below. More than 42 cell lines have been screened for TC1 gene expression by Northern Blot analysis. However, only two cell lines, JMN1B and JMN, express detectable mRNA by Northern Blot analysis. JMN1B and JMN are malignant mesothelioma, JMN1B being a subline of JMN cells with showing enhanced tumorigenicity after passage of JMN cells through a nude mouse. Fig. 25 is a Northern Blot analysis of RNA from malignant mesothelioma cells JMN1B and JMN using TC1 cDNA as a probe. The results presented in Panel 25B demonstrate that TC1 mRNA level in JMN1B cells (2) is much greater than that in JMN cells (1). Panel 25A shows the ethidium bromide

staining pattern of an RNA gel in which the same amount of JMN (1) and JMN1B (2) RNA is loaded per lane.

The Northern Blot analysis revealed that the TC1 mRNA level is much higher in JMN1B than in JMN, the JMN1B/JMN ratio being approximately 14. Higher expression of TC1 mRNA in JMN1B could be related to the observed greater tumorigenicity of JMN1B cells. It has been found that JMN1B cells can secrete an "EGF-like" growth factor called transformed mesothelial growth factor (TMGF) that satisfies the EGF requirement of normal human mesothelial cells. The difference in the levels of TC1 mRNA in JMN1B and JMN cells provides an ideal cell model to understand the regulation of TC1 expression and its relation to tumorigenicity.

Sequence analysis of the deduced amino acid sequence has revealed that the TC1 protein (SEQ ID NO:4) contained a secretory leader signal at its N-terminus. The secretion of TC1 protein was confirmed by Wester Blot analysis of conditioned medium of JMN1B cells. JMN1B cells were cultured in regular medium until 90% confluent, then cultured in serum free medium for two days. This serum free conditioned medium was analyzed by immunoblotting with anti-TC1 monoclonal antibody. Fig. 26 is a Western blot analysis using a monoclonal antibody against TC1 to probe JMN1B cells grown in conditioned medium and whole cell lysate. Two major bands (about 86kd and 104kd) were recognized by anti-TC1 antibody both in JMN1B cell conditioned medium (1) and whole cell lysate (2). Numbers on the left indicate the position of molecular weight standards in kilodalton. The protein size of the lower molecular weight 86kd band is consistent with that of deduced TC1 protein, whereas the higher molecular weight 104kd band is consistent with a TC1 glycoprotein. There is one predicted site of N-linked glycosylation at the amino acid residue 605(NDT) of deduced TC1 protein sequence. There are 60 threonine residues and 36 serine residues in the deduced TC1 sequence, each of which is a potential site of O-linked glycosylation.

Human malignant mesothelioma cell line JMN1B can express abundant TC1. This cell line was used to study the distribution and localization of TC1 protein by immunofluorescent staining with an anti-TC1 monoclonal antibody followed by Rhodamine conjugated goat anti-mouse IgG secondary antibody. When JMN1B cells were fixed with paraformaldehyde without subsequent permeabilization, the positive staining was seen on the cell surface or outside of the cell (Fig. 27, panels A,B), which confirms the secretion of TC1 protein. When JMN1B cells were fixed with paraformaldehyde and then permeabilized, positive staining appeared in the Golgi complex and the endoplasmic reticulum (ER) in the cell (Fig. 27, panels C,D), suggesting that TC1 protein is synthesized in the ER and Golgi complex. The staining in the Golgi complex is clearly evident, indicating that glycosylation of TC1 protein may be located in the Golgi complex. The TC1 protein distribution pattern also suggests that TC1 is a secreted glycoprotein.

Without being bound to one theory as to the biological function of TC1, observations as to the prevalence and expression of TC1 mRNA and protein indicate that TC1 may be related to tumor malignant behavior such as invasion and metastases. These observations include the following: TC1 is significantly overexpressed in tumor tissue; TC1 is a secreted protein; later stage tumor expresses higher levels of TC1; deeper invasive tumor contains higher levels of TC1 protein; TC1 expression turns off in cultured tumor cells *in vitro* and turns on again after cells grow tumor tissue *in vivo*. These observations indicate that the function of TC1 is not related to tumor cell proliferation, but is more likely involved in tumor malignant behavior *in vivo*, such as invasion and metastases.

TC1 is a member of a Family of Proteins.

A FASTA search of the GenBank and EMBL database with the TC1 open reading frame indicated that the protein is unique.

However, TC1 whole protein shared 45% sequence identity with a TGF-beta inducible gene, Big-h3, at the amino acid level, suggesting that TC1 and Big-h3 may belong to a new gene family. The identity between TC1 (SEQ ID NO:4) and Big-h3 (SEQ ID NO:11) at the amino acid level is shown in Fig. 7. In Fig. 7, identical amino acids between TC1 and Big-h3 are boxed. Several stretches of amino acids GSFTXFAPSNEAW, TLXAPTNEAFEKXP, ATNGVVHXIDXV, LYXGQXLETXGGKXLRVFVYR, HYPNGXVTVNCAR are highly conserved between TC1 and Big-h3. Northern Blot analysis showed that the TC1 gene is expressed from a larger transcript than Big-h3, and DNA sequence analysis indicated that TC1 contains a longer open reading frame encoding a higher molecular weight protein than the Big-h3 gene. It has been found that Big-h3 also contains four internal repeats. The amino acid sequence homology and structural similarity between TC1 and Big-h3 indicate their functional similarity and relationship. We found that Big-h3 mRNA is also much more abundant in colon carcinoma tissue than in adjacent normal colon tissue (Fig. 7). Fig. 8 is a Northern Blot of five pairs of RNA from colon carcinoma (T) and adjacent normal colon tissue (N) probed with ^{32}P-labeled Big-h3 cDNA. The blot shows Big-h3 mRNA level in colon carcinoma to be much higher than that in adjacent normal tissue. The bottom panel represents control RNA probed with ^{32}P-labeled B-actin.

In contrast to the expression pattern of TC1 mRNA, which is shown to be largely restricted to *in vivo* tumor tissue, Big-h3 mRNA is not only expressed in the tumor tissue, but also expressed in the cultured tumor cell lines and some normal cell lines. Though TC1 and Big-h3 shared significant homology, their responses to growth factors are distinctly different.

Fasciclin I, II, III are extrinsic membrane glycoproteins involved in the growth cone guidance during nervous system development in the insect embryo. A search of NBRF protein database revealed a significant homologous

domain between TC1 and Fasciclin I from Grasshopper and Drosophila. One TC1 domain of 204 amino acids (amino acid residue 503-706) shared 30% identity with Grasshopper Fasciclin I, and shared 25% identity with Drosophila Fasciclin. Fig. 9 shows amino acid sequence homology between TC1 (SEQ ID NO:4) and Fasciclin I from Grasshopper (GrF) (SEQ ID NO:18) and Drosophila (DrF) (SEQ ID NO:19). Boxed amino acids are identical with at least one other amino acid at that same position.

It has been found that Fasciclin also contained four internal homologous domains, each consisting of approximately 150 amino acids. The domains of TC1 and Fasciclin I share some highly conserved amino acid stretches such as TXF$\frac{V}{A}$PTNXAF, and VXHVVDXXLXP.

The most conserved sequence among TC1, Big-h3 and Fasciclin is TXF$\frac{A}{V}$PTNXA$\frac{F}{W}$. All four internal repeats in TC1 or Big-h3 or Fasciclin I also share the most conserved sequence TXF$\frac{A}{V}$P$\frac{T}{S}$NXA$\frac{F}{W}$. This sequence appears to be an important motif of this gene family.

<u>Screening for antagonists to TC1 function.</u>

The invention also includes methods of screening for agents which inhibit TC1 gene expression, whether such inhibition be at the transcriptional or translational level.

Screening methods, according to the invention, for agents which inhibit expression of the TC1 gene *in vitro* will include exposing a metastatic cell line in which TC1 mRNA is detectable in culture to an agent suspected of inhibiting production of the TC1 mRNA; and determining the level of TC1 mRNA in the exposed cell line, wherein a decrease in the level of TC1 mRNA after exposure of the cell line to the agent is indicative of inhibition of TC1 mRNA production.

Alternatively, such screening methods may include *in vitro* screening of a metastatic cell line in which TC1 protein is detectable in culture to an agent suspected of inhibiting production of the TC1 protein; and determining the level of TC1 protein in the cell line, wherein a decrease in the level of TC1 protein after exposure of the cell line to the agent is indicative of inhibition of TC1 protein production.

The invention also encompasses *in vivo* methods of screening for agents which inhibit expression of the TC1 gene, comprising

exposing a mammal having tumor cells in which TC1 mRNA or protein is detectable to an agent suspected of inhibiting production of TC1 mRNA or protein; and determining the level of TC1 mRNA or protein in tumor cells of the exposed mammal. A decrease in the level of TC1 mRNA or protein after exposure of the mammal to the agent is indicative of inhibition of TC1 gene expression.

According to the invention, agents can be screened *in vitro* or *in vivo* as follows. For *in vitro* screening, a metastatic cell line, e.g., JMN1B, may be cultured *in vitro* and exposed to an agent suspected of inhibiting TC1 expression in an amount and for a time sufficient to inhibit such expression. For *in vivo* screening, a mammal afflicted with a late stage cancer, particularly one of breast cancer, colon cancer, or cancer of the gastrointestinal tract, is exposed to the agent at a dosage and for a time sufficient to inhibit expression of TC1. A late stage cancer is defined by the Duke's stage of the cancer; i.e., late stage cancers correspond to Duke's stages 3-4. The amount or dosage of the agent which is effective to inhibit TC1 expression may be determined using serial dilutions of the agent. The level of TC1 mRNA or protein may be determined using an aliquot of cells from the cell culture or the *in vivo* tumor and performing Northern Blot analysis or Western Blot analysis, respectively. The agent will be considered inhibitory if the

level of TC1 mRNA or protein decreases by more than 50%, and preferably more than 70-80%, relative to the same cell line which has not been exposed to the agent.

Examples of potential inhibitors of TC1 mRNA or protein production include but are not limited to antisense RNA, competitive inhibitors of the TC1 protein such as fragments of the TC1 protein itself, or antibodies to TC1 protein. Candidate TC1 inhibitory fragments include, but are not limited to, $\mathrm{T}\frac{L}{Y}\mathrm{F}\frac{A}{V}\mathrm{P}\frac{T}{S}\mathrm{N}\frac{E}{D}\mathrm{A}\frac{F}{W}$ and $\mathrm{NG}\frac{V}{A}\frac{I}{V}\mathrm{HX}\frac{I}{V}\frac{D}{F}$.

Use of anatagonists to TC1 functions.

The invention also encompasses the treatment of late stage cancers by administration to a mammal afflicted with a late stage cancer one or more of the above-selected inhibitory agents. Late stage cancers, particularly those of the breast, colon, or gastrointestinal tract, are treated according to the invention by administering the inhibitory agent to a mammal afflicted with a late stage cancer in an amount and for a time sufficient to decrease the level of TC1 protein or mRNA.

The mode of administration may be intravenously, intraperitoneally, by intramuscular or intradermal injection, or orally. Administration may be by single dose, or may be continuous or intermittent. The dosage of inhibitory agent is that dosage which is effective to inhibit TC1 production, i.e., within the range of 10 μg/kg body weight - 100 gm/kg body weight, preferably, within the range of 1 mg/kg body weight - 1 gm/kg body weight, most preferably 10-100 mg/kg body weight.

Production of monoclonal antibodies reactive with TC1.

An anti-TC1 antibody is produced according to Kohler and Milstein, Nature, 256:495-497 (1975), Eur. J. Immunol. 6:511-519 (1976), both of which are hereby incorporated by reference, using the TC1 protein or a fragment thereof as the

immunizing antigen. Hybridomas produced by the above process are selected for anti-TC1 antibodies using the TC1 as an antigen in an ELISA assay. The single type of immunoglobulin produced by a such a hybridoma is specific for a single antigenic determinant, or epitope, on the TC1 antigen. Certain TC1-specific antibodies, for example, anti-TC1-1 produced by the hybridoma deposited with the American Type Culture Collection (ATCC) under the ATCC number HB 11481, are unique in that they recognize the TC1 protein, more specifically an epitope of the TC1 protein, in formaldehyde-fixed and paraffin-embedded tumor cells which bear TC1.

Deposits

The following samples were deposited on October 29, 1993, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852.

Deposit	ATCC Accession No.
TC1 gene in pBluescript plasmid DNA vector	75599
Hybridoma TC-1	HB 11481

Applicants' assignee, Dana-Farber Cancer Institute, Inc., represents that the ATCC is a depository affording permanence of the deposit and ready accessibility thereto by the public if a patent is granted. All restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent. The material will be available during the pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR 1,14 and 35 USC 122. The deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited microorganism, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the

patent, whichever period is longer. Applicants' assignee acknowledges its duty to replace the deposit should the depository be unable to furnish a sample when requested due to the condition of the deposit.

OTHER EMBODIMENTS

The invention is not limited to those embodiments described herein, but may encompass modifications and variations which do not depart from the spirit of the invention. While the invention has been described in connection with specific embodiments thereof, it will be understood that further modifications are within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Chen, Lan Bo
Bao, Shideng
Liu, Yuan

(ii) TITLE OF INVENTION: A NOVEL TUMOR MARKER AND NOVEL METHOD OF ISOLATING SAME

(iii) NUMBER OF SEQUENCES: 19

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Weingarten, Schurgin, Gagnebin & Hayes
(B) STREET: Ten Post Office Square
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/146,488
(B) FILING DATE: 29-OCT-1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Heine, Holliday C.
(B) REGISTRATION NUMBER: 34,346
(C) REFERENCE/DOCKET NUMBER: DFCI-333XX

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 543-2290
(B) TELEFAX: (617) 451-0313

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 636 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..636

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GAC CGT GTG CTT ACA CAA ATT GGT ACC TCA ATT CAA GAC TTC ATT GAA      96
Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln Asp Phe Ile Glu
             20                  25                  30
GCA GAA GAT GAC CTT TCA TCT TTT AGA GCA GCT GCC ATC ACA TCG GAC     144
Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr Ser Asp
         35                  40                  45
ATA TTG GAG GCC CTT GGA AGA GAC GGT CAC TTC ACA CTC TTT GCT CCC     192
Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe Ala Pro
     50                  55                  60
ACC AAT GAG GCT TTT GAG AAA CTT CCA CGA GGT GTC CTA GAA AGG ATC     240
Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu Arg Ile
 65                  70                  75                  80
ATG GGA GAC AAA GTG GCT TCC GAA GCT CTT ATG AAG TAC CAC ATC TTA     288
Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His Ile Leu
                 85                  90                  95
AAT ACT CTC CAG TGT TCT GAG TCT ATT ATG GGA GGA GCA GTC TTT GAG     336
Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val Phe Glu
            100                 105                 110
ACG CTG GAA GGA AAT ACA ATT GAG ATA GGA TGT GAC GGT GAC AGT ATA     384
Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp Ser Ile
        115                 120                 125
ACA GTA AAT GGA ATC AAA ATG GTG AAC AAA AAG GAT ATT GTG ACA AAT     432
Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val Thr Asn
    130                 135                 140
AAT GGT GTG ATC CAT TTG ATT GAT CAG GTC CTA ATT CCT GAT TCT GCC     480
Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp Ser Ala
145                 150                 155                 160
AAA CAA GTT ATT GAG CTG GCT GGA AAA CAG CAA ACC ACC TTC ACG GAT     528
Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe Thr Asp
                165                 170                 175
CTT GTG GCC CAA TTA GGC TTG GCA TCT GCT CTG AGG CCA GAT GGA GAA     576
Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp Gly Glu
            180                 185                 190
TAC ACT TTG CTG GCA CCT GTG AAT AAT GCA TTT TCT GAT GAT ACT CTC     624
Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp Thr Leu
        195                 200                 205
AGC ATG GAT CAG                                                     636
Ser Met Asp Gln
    210
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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             20                  25                  30
Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala Ile Thr Ser Asp
         35                  40                  45
Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr Leu Phe Ala Pro
     50                  55                  60
Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val Leu Glu Arg Ile
 65                  70                  75                  80
Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys Tyr His Ile Leu
                 85                  90                  95
Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly Ala Val Phe Glu
            100                 105                 110
Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp Gly Asp Ser Ile
        115                 120                 125
Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp Ile Val Thr Asn
    130                 135                 140
Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile Pro Asp Ser Ala
145                 150                 155                 160
Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr Thr Phe Thr Asp
                165                 170                 175
Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg Pro Asp Gly Glu
            180                 185                 190
Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser Asp Asp Thr Leu
        195                 200                 205
Ser Met Asp Gln
    210
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3126 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 43..2376

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GCCACCATGT AGCCCGCGTC ACCGTTCTGC GCATTCCGCA GC ATG GCT CTG CCT      54
                                               Met Ala Leu Pro
                                                1

GCC CGA ATC CTC GCT CTG GCC CTC GCA CTG GCG CTC GGA CCC GCC GTG     102
Ala Arg Ile Leu Ala Leu Ala Leu Ala Leu Ala Leu Gly Pro Ala Val
  5                  10                  15                  20

ACA CTG GCC AAC CCG GCG AGA ACG CCG TAC GAG CTG GTA CTC CAG AAG     150
Thr Leu Ala Asn Pro Ala Arg Thr Pro Tyr Glu Leu Val Leu Gln Lys
                 25                  30                  35

AGC TCG GCA CGA GGG GGT CGG GAC CAA GGC CCA AAT GTC TGT GCC CTT     198
Ser Ser Ala Arg Gly Gly Arg Asp Gln Gly Pro Asn Val Cys Ala Leu
             40                  45                  50

CAA CAG ATT TTG GGC ACC AAA AAG AAA TAC TTC AGC ACT TGT AAG AAC     246
Gln Gln Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser Thr Cys Lys Asn
         55                  60                  65

TGG TAT AAA AAG TCC ATC TGT GGA CAG AAA ACG ACT GTG TTA TAT GAA     294
Trp Tyr Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr Val Leu Tyr Glu
     70                  75                  80

TGT TGC CCT GGT TAT ATG AGA ATG GAA GGA ATG AAA GGC TGC CCA GCA     342
Cys Cys Pro Gly Tyr Met Arg Met Glu Gly Met Lys Gly Cys Pro Ala
 85                  90                  95                 100

GTT TTG CCC ATT GAC CAT GTT TAT GGC ACT CTG GGC ATC GTG GGA GCC     390
Val Leu Pro Ile Asp His Val Tyr Gly Thr Leu Gly Ile Val Gly Ala
                105                 110                 115

ACC ACA ACG CAG CGC TAT TCT GAC GCC TCA AAA CTG AGG GAG GAG ATC     438
Thr Thr Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu Arg Glu Glu Ile
            120                 125                 130

GAG GGA AAG GGA TCC TTC ACT TAC TTT GCA CCG AGT AAT GAG GCT TGG     486
Glu Gly Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser Asn Glu Ala Trp
        135                 140                 145

GAC AAC TTG GAT TCT GAT ATC CGT AGA GGT TTG GAG AGC AAC GTG AAT     534
Asp Asn Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu Ser Asn Val Asn
    150                 155                 160

GTT GAA TTA CTG AAT GCT TTA CAT AGT CAC ATG ATT AAT AAG AGA ATG     582
Val Glu Leu Leu Asn Ala Leu His Ser His Met Ile Asn Lys Arg Met
165                 170                 175                 180

TTG ACC AAG GAC TTA AAA AAT GGC ATG ATT ATT CCT TCA ATG TAT AAC     630
Leu Thr Lys Asp Leu Lys Asn Gly Met Ile Ile Pro Ser Met Tyr Asn
                185                 190                 195
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AAT TTG GGG CTT TTC ATT AAC CAT TAT CCT AAT GGG GTT GTC ACT GTT      678
Asn Leu Gly Leu Phe Ile Asn His Tyr Pro Asn Gly Val Val Thr Val
            200                 205                 210
AAT TGT GCT CGA ATC ATC CAT GGG AAC CAG ATT GCA ACA AAT GGT GTT      726
Asn Cys Ala Arg Ile Ile His Gly Asn Gln Ile Ala Thr Asn Gly Val
        215                 220                 225
GTC CAT GTC ATT GAC CGT GTG CTT ACA CAA ATT GGT ACC TCA ATT CAA      774
Val His Val Ile Asp Arg Val Leu Thr Gln Ile Gly Thr Ser Ile Gln
    230                 235                 240
GAC TTC ATT GAA GCA GAA GAT GAC CTT TCA TCT TTT AGA GCA GCT GCC      822
Asp Phe Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe Arg Ala Ala Ala
245                 250                 255                 260
ATC ACA TCG GAC ATA TTG GAG GCC CTT GGA AGA GAC GGT CAC TTC ACA      870
Ile Thr Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp Gly His Phe Thr
                265                 270                 275
CTC TTT GCT CCC ACC AAT GAG GCT TTT GAG AAA CTT CCA CGA GGT GTC      918
Leu Phe Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu Pro Arg Gly Val
            280                 285                 290
CTA GAA AGG ATC ATG GGA GAC AAA GTG GCT TCC GAA GCT CTT ATG AAG      966
Leu Glu Arg Ile Met Gly Asp Lys Val Ala Ser Glu Ala Leu Met Lys
        295                 300                 305
TAC CAC ATC TTA AAT ACT CTC CAG TGT TCT GAG TCT ATT ATG GGA GGA     1014
Tyr His Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser Ile Met Gly Gly
    310                 315                 320
GCA GTC TTT GAG ACG CTG GAA GGA AAT ACA ATT GAG ATA GGA TGT GAC     1062
Ala Val Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu Ile Gly Cys Asp
325                 330                 335                 340
GGT GAC AGT ATA ACA GTA AAT GGA ATC AAA ATG GTG AAC AAA AAG GAT     1110
Gly Asp Ser Ile Thr Val Asn Gly Ile Lys Met Val Asn Lys Lys Asp
                345                 350                 355
ATT GTG ACA AAT AAT GGT GTG ATC CAT TTG ATT GAT CAG GTC CTA ATT     1158
Ile Val Thr Asn Asn Gly Val Ile His Leu Ile Asp Gln Val Leu Ile
            360                 365                 370
CCT GAT TCT GCC AAA CAA GTT ATT GAG CTG GCT GGA AAA CAG CAA ACC     1206
Pro Asp Ser Ala Lys Gln Val Ile Glu Leu Ala Gly Lys Gln Gln Thr
        375                 380                 385
ACC TTC ACG GAT CTT GTG GCC CAA TTA GGC TTG GCA TCT GCT CTG AGG     1254
Thr Phe Thr Asp Leu Val Ala Gln Leu Gly Leu Ala Ser Ala Leu Arg
    390                 395                 400
CCA GAT GGA GAA TAC ACT TTG CTG GCA CCT GTG AAT AAT GCA TTT TCT     1302
Pro Asp Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn Asn Ala Phe Ser
405                 410                 415                 420
```

GAT GAT ACT CTC AGC ATG GAT CAG CGC CTC CTT AAA TTA ATT CTG CAG 1350
Asp Asp Thr Leu Ser Met Asp Gln Arg Leu Leu Lys Leu Ile Leu Gln
425 430 435

AAT CAC ATA TTG AAA GTA AAA GTT GGC CTT AAT GAG CTT TAC AAC GGG 1398
Asn His Ile Leu Lys Val Lys Val Gly Leu Asn Glu Leu Tyr Asn Gly
440 445 450

CAA ATA CTG GAA ACC ATC GGA GGC AAA CAG CTC AGA GTC TTC GTA TAT 1446
Gln Ile Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg Val Phe Val Tyr
455 460 465

CGT ACA GCT GTC TGC ATT GAA AAT TCA TGC ATG GAG AAA GGG AGT AAG 1494
Arg Thr Ala Val Cys Ile Glu Asn Ser Cys Met Glu Lys Gly Ser Lys
470 475 480

CAA GGG AGA AAC GGT GCG ATT CAC ATA TTC CGC GAG ATC ATC AAG CCA 1542
Gln Gly Arg Asn Gly Ala Ile His Ile Phe Arg Glu Ile Ile Lys Pro
485 490 495 500

GCA GAG AAA TCC CTC CAT GAA AAG TTA AAA CAA GAT AAG CGC TTT ACG 1590
Ala Glu Lys Ser Leu His Glu Lys Leu Lys Gln Asp Lys Arg Phe Thr
505 510 515

ACC TTC CTC AGC CTA CTT GAA GCT GCA GAC TTG AAA GAG CTC CTG ACA 1638
Thr Phe Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys Glu Leu Leu Thr
520 525 530

CAA CCT GGA GAC TGG ACA TTA TTT GTG CCA ACC AAT GAT GCT TTT AAG 1686
Gln Pro Gly Asp Trp Thr Leu Phe Val Pro Thr Asn Asp Ala Phe Lys
535 540 545

GGA ATG ACT AGT GAA GAA AAA GAA ATT CTG ATA CGG GAC AAA AAT GCT 1734
Gly Met Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg Asp Lys Asn Ala
550 555 560

CTT CAA AAC ATC ATT CTT TAT CAC CTG ACA CCA GGA GTT TTC ATT GGA 1782
Leu Gln Asn Ile Ile Leu Tyr His Leu Thr Pro Gly Val Phe Ile Gly
565 570 575 580

AAA GGA TTT GAA CCT GGT GTT ACT AAC ATT TTA AAG ACC ACA CAA GGA 1830
Lys Gly Phe Glu Pro Gly Val Thr Asn Ile Leu Lys Thr Thr Gln Gly
585 590 595

AGC AAA ATC TTT CTG AAA GAA GTA AAT GAT ACA CTT CTG GTG AAT GAA 1878
Ser Lys Ile Phe Leu Lys Glu Val Asn Asp Thr Leu Leu Val Asn Glu
600 605 610

TTG AAA TCA AAA GAA TCT GAC ATC ATG ACA ACA AAT GGT GTA ATT CAT 1926
Leu Lys Ser Lys Glu Ser Asp Ile Met Thr Thr Asn Gly Val Ile His
615 620 625

GTT GTA GAT AAA CTC CTC TAT CCA GCA GAC ACA CCT GTT GGA AAT GAT 1974
Val Val Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro Val Gly Asn Asp
630 635 640

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CAA CTG CTG GAA ATA CTT AAT AAA TTA ATC AAA TAC ATC CAA ATT AAG      2022
Gln Leu Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr Ile Gln Ile Lys
645                 650                 655                 660
TTT GTT CGT GGT AGC ACC TTC AAA GAA ATC CCC GTG ACT GTC TAT AGA      2070
Phe Val Arg Gly Ser Thr Phe Lys Glu Ile Pro Val Thr Val Tyr Arg
                665                 670                 675
CCC ACA CTA ACA AAA GTC AAA ATT GAA GGT GAA CCT GAA TTC AGA CTG      2118
Pro Thr Leu Thr Lys Val Lys Ile Glu Gly Glu Pro Glu Phe Arg Leu
            680                 685                 690
ATT AAA GAA GGT GAA ACA ATA ACT GAA GTG ATC CAT GGA GAG CCA ATT      2166
Ile Lys Glu Gly Glu Thr Ile Thr Glu Val Ile His Gly Glu Pro Ile
        695                 700                 705
ATT AAA AAA TAC ACC AAA ATC ATT GAT GGA GTG CCT GTG GAA ATA ACT      2214
Ile Lys Lys Tyr Thr Lys Ile Ile Asp Gly Val Pro Val Glu Ile Thr
    710                 715                 720
GAA AAA GAG ACA CGA GAA GAA CGA ATC ATT ACA GGT CCT GAA ATA AAA      2262
Glu Lys Glu Thr Arg Glu Glu Arg Ile Ile Thr Gly Pro Glu Ile Lys
725                 730                 735                 740
TAC ACT AGG ATT TCT ACT GGA GGT GGA GAA ACA GAA GAA ACT CTG AAG      2310
Tyr Thr Arg Ile Ser Thr Gly Gly Gly Glu Thr Glu Glu Thr Leu Lys
                745                 750                 755
AAA TTG TTA CAA GAA GAC ACA CCC GTG AGG AAG TTG CAA GCC AAC AAA      2358
Lys Leu Leu Gln Glu Asp Thr Pro Val Arg Lys Leu Gln Ala Asn Lys
            760                 765                 770
AAA AGT TCA AGG ATC TAGAAGACGA TTAAGGGAAG GTCGTTCTCA GTGAAAATCC      2413
Lys Ser Ser Arg Ile
        775
AAAAACCAGA AAAAAATGTT TATACAACCC TAAGTCAATA ACCTGACCTT AGAAAATTGT    2473

GAGAGCCAAG TTGACTTCAG GAACTGAAAC ATCAGCACAA AGAAGCAATC ATCAAATAAT    2533
TCTGAACACA AATTTAATAT TTTTTTTTCT GAATGAGAAA CATGAGGGAA ATTGTGGAGT    2593
TAGCCTCCTG TGGTAAAGGA ATTGAAGAAA ATATAACACC TTACACCCTT TTTCATCTTG    2653
ACATTAAAAG TTCTGGCTAA CTTTGGAATC CATTAGAGAA AAATCCTTGT CACCAGATTC    2713
ATTACAATTC AAATCGAAGA GTTGTGAACT GTTATCCCAT TGAAAAGACC GAGCCTTGTA    2773
TGTATGTTAT GGATACATAA AATGCACGCA AGCCATTATC TCTCCATGGG AAGCTAAGTT    2833
ATAAAAATAG GTGCTTGGTG TACAAAACTT TTTATGATCA AAAGGCTTTG CACATTTCTA    2893
TATGAGTGGG TTTACTGGTA AATTATGTTA TTTTTTACAA CTAATTTTGT ACTCTCAGAA    2953
TGTTTGTCAT ATGCTTCTTG CAATGCATAT TTTTTAATCT CAAACGTTTC AATAAAACCA    3013
TTTTTCAGAT ATAAAGAGAA TTACTTCAAA TTGAGTAATT CAGAAAAACT CAAGATTTAA    3073
GTTAAAAAGT GGTTTGGACT TGGGAATAGG ACTTTATACC TCTTTCTCGT GCC           3126
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 777 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Leu Pro Ala Arg Ile Leu Ala Leu Ala Leu Ala Leu Ala Leu
 1               5                  10                  15
Gly Pro Ala Val Thr Leu Ala Asn Pro Ala Arg Thr Pro Tyr Glu Leu
            20                  25                  30
Val Leu Gln Lys Ser Ser Ala Arg Gly Gly Arg Asp Gln Gly Pro Asn
        35                  40                  45
Val Cys Ala Leu Gln Gln Ile Leu Gly Thr Lys Lys Lys Tyr Phe Ser
    50                  55                  60
Thr Cys Lys Asn Trp Tyr Lys Lys Ser Ile Cys Gly Gln Lys Thr Thr
65                  70                  75                  80
Val Leu Tyr Glu Cys Cys Pro Gly Tyr Met Arg Met Glu Gly Met Lys
                85                  90                  95
Gly Cys Pro Ala Val Leu Pro Ile Asp His Val Tyr Gly Thr Leu Gly
            100                 105                 110
Ile Val Gly Ala Thr Thr Thr Gln Arg Tyr Ser Asp Ala Ser Lys Leu
        115                 120                 125
Arg Glu Glu Ile Glu Gly Lys Gly Ser Phe Thr Tyr Phe Ala Pro Ser
    130                 135                 140
Asn Glu Ala Trp Asp Asn Leu Asp Ser Asp Ile Arg Arg Gly Leu Glu
145                 150                 155                 160
Ser Asn Val Asn Val Glu Leu Leu Asn Ala Leu His Ser His Met Ile
                165                 170                 175
Asn Lys Arg Met Leu Thr Lys Asp Leu Lys Asn Gly Met Ile Ile Pro
            180                 185                 190
Ser Met Tyr Asn Asn Leu Gly Leu Phe Ile Asn His Tyr Pro Asn Gly
        195                 200                 205
Val Val Thr Val Asn Cys Ala Arg Ile Ile His Gly Asn Gln Ile Ala
    210                 215                 220
Thr Asn Gly Val Val His Val Ile Asp Arg Val Leu Thr Gln Ile Gly
225                 230                 235                 240
Thr Ser Ile Gln Asp Phe Ile Glu Ala Glu Asp Asp Leu Ser Ser Phe
                245                 250                 255
Arg Ala Ala Ala Ile Thr Ser Asp Ile Leu Glu Ala Leu Gly Arg Asp
            260                 265                 270
Gly His Phe Thr Leu Phe Ala Pro Thr Asn Glu Ala Phe Glu Lys Leu
        275                 280                 285
Pro Arg Gly Val Leu Glu Arg Ile Met Gly Asp Lys Val Ala Ser Glu
    290                 295                 300
Ala Leu Met Lys Tyr His Ile Leu Asn Thr Leu Gln Cys Ser Glu Ser
305                 310                 315                 320
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Ile Met Gly Gly Ala Val Phe Glu Thr Leu Glu Gly Asn Thr Ile Glu
                325                 330                 335
Ile Gly Cys Asp Gly Asp Ser Ile Thr Val Asn Gly Ile Lys Met Val
            340                 345                 350
Asn Lys Lys Asp Ile Val Thr Asn Asn Gly Val Ile His Leu Ile Asp
        355                 360                 365
Gln Val Leu Ile Pro Asp Ser Ala Lys Gln Val Ile Glu Leu Ala Gly
    370                 375                 380
Lys Gln Gln Thr Thr Phe Thr Asp Leu Val Ala Gln Leu Gly Leu Ala
385                 390                 395                 400
Ser Ala Leu Arg Pro Asp Gly Glu Tyr Thr Leu Leu Ala Pro Val Asn
                405                 410                 415
Asn Ala Phe Ser Asp Asp Thr Leu Ser Met Asp Gln Arg Leu Leu Lys
            420                 425                 430
Leu Ile Leu Gln Asn His Ile Leu Lys Val Lys Val Gly Leu Asn Glu
        435                 440                 445
Leu Tyr Asn Gly Gln Ile Leu Glu Thr Ile Gly Gly Lys Gln Leu Arg
    450                 455                 460
Val Phe Val Tyr Arg Thr Ala Val Cys Ile Glu Asn Ser Cys Met Glu
465                 470                 475                 480
Lys Gly Ser Lys Gln Gly Arg Asn Gly Ala Ile His Ile Phe Arg Glu
                485                 490                 495
Ile Ile Lys Pro Ala Glu Lys Ser Leu His Glu Lys Leu Lys Gln Asp
            500                 505                 510
Lys Arg Phe Thr Thr Phe Leu Ser Leu Leu Glu Ala Ala Asp Leu Lys
        515                 520                 525
Glu Leu Leu Thr Gln Pro Gly Asp Trp Thr Leu Phe Val Pro Thr Asn
    530                 535                 540
Asp Ala Phe Lys Gly Met Thr Ser Glu Glu Lys Glu Ile Leu Ile Arg
545                 550                 555                 560
Asp Lys Asn Ala Leu Gln Asn Ile Ile Leu Tyr His Leu Thr Pro Gly
                565                 570                 575
Val Phe Ile Gly Lys Gly Phe Glu Pro Gly Val Thr Asn Ile Leu Lys
            580                 585                 590
Thr Thr Gln Gly Ser Lys Ile Phe Leu Lys Glu Val Asn Asp Thr Leu
        595                 600                 605
Leu Val Asn Glu Leu Lys Ser Lys Glu Ser Asp Ile Met Thr Thr Asn
    610                 615                 620
Gly Val Ile His Val Val Asp Lys Leu Leu Tyr Pro Ala Asp Thr Pro
625                 630                 635                 640
Val Gly Asn Asp Gln Leu Leu Glu Ile Leu Asn Lys Leu Ile Lys Tyr
                645                 650                 655
Ile Gln Ile Lys Phe Val Arg Gly Ser Thr Phe Lys Glu Ile Pro Val
            660                 665                 670
```

```
Thr Val Tyr Arg Pro Thr Leu Thr Lys Val Lys Ile Glu Gly Glu Pro
        675                 680                 685
Glu Phe Arg Leu Ile Lys Glu Gly Glu Thr Ile Thr Glu Val Ile His
    690                 695                 700
Gly Glu Pro Ile Ile Lys Lys Tyr Thr Lys Ile Ile Asp Gly Val Pro
705                 710                 715                 720
Val Glu Ile Thr Glu Lys Glu Thr Arg Glu Glu Arg Ile Ile Thr Gly
                725                 730                 735
Pro Glu Ile Lys Tyr Thr Arg Ile Ser Thr Gly Gly Gly Glu Thr Glu
            740                 745                 750
Glu Thr Leu Lys Lys Leu Leu Gln Glu Asp Thr Pro Val Arg Lys Leu
        755                 760                 765
Gln Ala Asn Lys Lys Ser Ser Arg Ile
    770                 775
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGTCCAGATG

10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGTCCAGATG C

11

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGTCCAGATG AC
12

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TGTCCAGATA
10

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGTCCAGACG
10

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
TGTCCAGCCG
10

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
TGTCCCGCCG
10

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
TGCCCGGCCG
10

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
TGATGCACTC
10

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
TGAGCTACTC
10

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
TGACTGACTC
10

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
CTGATCCATG
10

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 683 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Met Ala Leu Phe Val Arg Leu Leu Ala Leu Ala Leu Ala Leu Ala Leu
1               5                   10                  15
Gly Pro Ala Ala Thr Leu Ala Gly Pro Ala Lys Ser Pro Tyr Gln Leu
            20                  25                  30
Pro Leu Gln His Ser Arg Leu Arg Gly Arg Gln His Gly Pro Asn Val
        35                  40                  45
Cys Ala Val Thr Lys Val Ile Gly Thr Asn Arg Lys Tyr Phe Thr Asn
    50                  55                  60
Cys Lys Gln Trp Tyr Gln Arg Lys Ile Cys Gly Lys Ser Thr Val Ile
65                  70                  75                  80
Ser Tyr Glu Cys Cys Pro Gly Tyr Glu Lys Val Pro Gly Glu Lys Gly
                85                  90                  95
Cys Pro Ala Ala Leu Pro Leu Ser Asn Leu Tyr Glu Thr Leu Gly Val
            100                 105                 110
Val Gly Ser Thr Thr Thr Gln Leu Tyr Thr Asp Arg Thr Glu Lys Leu
        115                 120                 125
Arg Pro Glu Met Glu Gly Pro Gly Ser Phe Thr Ile Phe Ala Pro Ser
    130                 135                 140
Asn Glu Ala Trp Ala Ser Leu Pro Ala Glu Val Leu Val Ser Leu Val
145                 150                 155                 160
Ser Asn Val Asn Ile Glu Leu Leu Asn Ala Leu Arg Tyr His Met Val
                165                 170                 175
Gly Arg Arg Val Leu Thr Asp Glu Leu Lys His Gly Met Thr Leu Thr
            180                 185                 190
Ser Met Tyr Gln Asn Ser Asn Ile Gln Ile His His Tyr Pro Asn Gly
        195                 200                 205
Ile Val Thr Val Asn Cys Ala Arg Leu Leu Lys Ala Asp His His Ala
    210                 215                 220
Thr Asn Gly Val Val His Leu Ile Asp Lys Val Ile Ser Thr Ile Thr
225                 230                 235                 240
Asn Asn Ile Gln Gln Ile Ile Glu Ile Glu Asp Thr Phe Glu Thr Leu
                245                 250                 255
Arg Ala Ala Val Ala Ala Ser Gly Leu Asn Thr Met Leu Glu Gly Asn
            260                 265                 270
Gly Gln Tyr Thr Leu Leu Ala Pro Thr Asn Glu Ala Phe Glu Lys Ile
        275                 280                 285
Pro Ser Glu Thr Leu Asn Arg Ile Leu Gly Asp Pro Glu Ala Leu Arg
    290                 295                 300
```

Asp Leu Leu Asn Asn His Ile Leu Lys Ser Ala Met Cys Ala Glu Ala
305 310 315 320
Ile Val Ala Gly Leu Ser Val Glu Thr Leu Glu Gly Thr Thr Leu Glu
325 330 335
Val Gly Cys Ser Gly Asp Met Leu Thr Ile Asn Gly Lys Ala Ile Ile
340 345 350
Ser Asn Lys Asp Ile Leu Ala Thr Asn Gly Val Ile His Tyr Ile Asp
355 360 365
Glu Leu Leu Ile Pro Asp Ser Ala Lys Thr Leu Phe Glu Leu Ala Ala
370 375 380
Glu Ser Asp Val Ser Thr Ala Ile Asp Leu Phe Arg Gln Ala Gly Leu
385 390 395 400
Gly Asn His Leu Ser Gly Ser Glu Arg Leu Thr Leu Leu Ala Pro Leu
405 410 415
Asn Ser Val Phe Lys Asp Gly Thr Pro Pro Ile Asp Ala His Thr Arg
420 425 430
Asn Leu Leu Arg Asn His Ile Ile Lys Asp Gln Leu Ala Ser Lys Tyr
435 440 445
Leu Tyr His Gly Gln Thr Leu Glu Thr Leu Gly Gly Lys Lys Leu Arg
450 455 460
Val Phe Val Tyr Arg Asn Ser Leu Cys Ile Glu Asn Ser Cys Ile Ala
465 470 475 480
Ala His Asp Lys Arg Gly Arg Tyr Gly Thr Leu Phe Thr Met Asp Arg
485 490 495
Val Leu Thr Pro Pro Met Gly Thr Val Met Asp Val Leu Lys Gly Asp
500 505 510
Asn Arg Phe Ser Met Leu Val Ala Ala Ile Gln Ser Ala Gly Leu Thr
515 520 525
Glu Thr Leu Asn Arg Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn
530 535 540
Glu Ala Phe Arg Ala Leu Pro Pro Arg Glu Ser Arg Arg Leu Leu Gly
545 550 555 560
Asp Ala Lys Glu Leu Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu
565 570 575
Ile Leu Val Ser Gly Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu
580 585 590
Gln Gly Asp Lys Leu Glu Val Ser Leu Lys Asn Asn Val Val Ser Val
595 600 605
Asn Lys Glu Pro Val Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val
610 615 620
Val His Val Ile Thr Asn Val Leu Gln Pro Pro Ala Asn Arg Pro Gln
625 630 635 640
Glu Arg Gly Asp Glu Leu Ala Asp Ser Ala Leu Glu Ile Phe Lys Gln
645 650 655

```
Ala Ser Ala Phe Ser Arg Ala Ser Gln Arg Ser Val Arg Leu Ala Val
            660                 665                 670
Pro Tyr Gln Lys Leu Leu Glu Arg Met Lys His
        675                 680
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 206 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Gly Glu Lys Ser Leu Glu Tyr Lys Ile Arg Asp Asp Pro Asp Leu Ser
1               5                   10                  15
Gln Phe Tyr Ser Trp Leu Glu His Asn Glu Val Ala Asn Ser Thr Leu
            20                  25                  30
Gln Leu Arg Gln Val Thr Val Phe Ala Pro Thr Asn Leu Ala Gln Phe
        35                  40                  45
Asn Tyr Lys Ala Arg Asp Gly Asp Glu Asn Ile Ile Leu Tyr His Met
    50                  55                  60
Thr Asn Leu Ala His Ser Leu Asp Gln Leu Gly His Lys Val Asn Ser
65                  70                  75                  80
Glu Leu Asp Gly Asn Pro Pro Leu Trp Ile Thr Arg Arg Arg Asp Thr
                85                  90                  95
Ile Phe Val Asn Asn Ala Arg Val Leu Thr Glu Arg Ser Asn Tyr Glu
            100                 105                 110
Ala Val Asn Arg His Gly Lys Lys Gln Val Leu His Val Val Asp Ser
        115                 120                 125
Val Leu Glu Pro Val Trp Ser Thr Ser Gly Gln Leu Tyr Asn Pro Asp
    130                 135                 140
Ala Phe Gln Phe Leu Asn Gln Ser Glu Asn Leu Asp Leu Gly Leu His
145                 150                 155                 160
Arg Val Arg Ser Phe Arg Gln Arg Val Phe Gln Asn Gln Lys Gln Asn
                165                 170                 175
Asp Phe Lys Leu Glu Gly Lys His Thr Phe Phe Ile Pro Val Asp Glu
            180                 185                 190
Gly Phe Lys Pro Leu Pro Arg Pro Glu Lys Ile Asp Gln Lys
        195                 200                 205
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 200 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Ala Ala Ala Asp Leu Ala Asp Lys Leu Arg Asp Asp Ser Glu Leu Ser
1               5                   10                  15
Gln Phe Tyr Ser Leu Leu Glu Ser Asn Gln Ile Ala Asn Ser Thr Leu
            20                  25                  30
Ser Leu Arg Ser Cys Thr Ile Phe Val Pro Thr Asn Glu Ala Phe Gln
        35                  40                  45
Arg Tyr Lys Ser Lys Thr Ala His Val Leu Tyr His Ile Thr Thr Glu
    50                  55                  60
Ala Tyr Thr Gln Lys Arg Leu Pro Asn Thr Val Ser Ser Asp Met Ala
65                  70                  75                  80
Gly Asn Pro Pro Leu Tyr Ile Thr Lys Asn Ser Asn Gly Asp Ile Phe
                85                  90                  95
Val Gly Asn Ala Arg Ile Ile Pro Ser Leu Ser Val Glu Thr Asn Ser
            100                 105                 110
Asp Gly Lys Arg Gln Ile Met His Ile Ile Asp Glu Val Leu Glu Pro
        115                 120                 125
Leu Thr V-` Lys Ala Gly His Ser Asp Thr Pro Asn Asn Pro Asn Ala
    130                 135                 140
Leu Lys Phe Leu Lys Asn Ala Glu Glu Phe Asn Val Asp Asn Ile Gly
145                 150                 155                 160
Val Arg Thr Tyr Arg Ser Gln Val Thr Met Ala Lys Lys Glu Ser Val
                165                 170                 175
Tyr Asp Ala Ala Gly Gln His Thr Phe Leu Val Pro Val Asp Glu Gly
            180                 185                 190
Phe Lys Leu Ser Ala Arg Ser Ser
        195                 200
```

CLAIMS

What is claimed is:

1. A monoclonal antibody that binds to an epitope of TC1 in formalin-fixed or paraffin-embedded tissues.

2. A monoclonal antibody produced by the hybridoma cell line ATCC No. HB 11481, or a monoclonal antibody that binds to the same antigenic determinant as a monoclonal antibody produced by the hybridoma cell line ATCC No. HB 11481.

3. A method of screening for agents that inhibit expression of the TC1 gene *in vitro*, comprising

exposing a metastatic cell line in which TC1 mRNA is detectable in culture to an agent suspected of inhibiting production of said TC1 mRNA; and

determining the level of TC1 mRNA in said exposed cell line, wherein a decrease in the level of TC1 mRNA after exposure of said cell line to said agent is indicative of inhibition of said TC1 mRNA production.

4. A method of screening for agents that inhibit expression of the TC1 protein *in vitro*, comprising

exposing a metastatic cell line in which TC1 protein is detectable in culture to an agent suspected of inhibiting production of said TC1 protein; and

determining the level of TC1 protein in said exposed cell line, wherein a decrease in the level of TC1 protein after exposure of said cell line to said agent is indicative of inhibition of said TC1 protein production.

5. The method of claim 3, said cell line comprising JMN1B.

6. The method of claim 4, said cell line comprising JMN1B.

7. A method of screening for agents that inhibit expression of the TC1 gene *in vivo*, comprising

exposing a mammal having tumor cells in which TC1 mRNA is detectable to an agent suspected of inhibiting production of said TC1 mRNA; and

determining the level of TC1 mRNA in tumor cells of said exposed mammal, wnerein a decrease in the level of TC1 mRNA after exposure of said mammal to said agent is indicative of inhibition of said TC1 mRNA production.

8. A method of screening for agents that inhibit production of TC1 protein *in vivo*, comprising

exposing a mammal having tumor cells in which TC1 protein is detectable to an agent suspected of inhibiting production of said TC1 protein; and

determining the level of TC1 protein in tumor cells of said exposed mammal, wherein a decrease in the level of TC1 protein after exposure of said mammal to said agent is indicative of inhibition of said TC1 protein production.

9. The method of claim 7, wherein said tumor cells are breast tumor cells, colon tumor cells, or tumor cells of the gastrointestinal tract.

10. The method of claim 8, wherein said tumor cells are breast tumor cells, colon tumor cells, or tumor cells of the gastrointestinal tract.

11. A pharmaceutical composition for use in treating a late stage cancer comprising an effective amount of an inhibitor of TC1.

12. The pharmaceutical composition of claim 11 wherein said late stage cancer is one of breast cancer, colon cancer, or gastrointestinal cancer.

13. A pharmaceutical composition for use in preventing tumor cell metastasis comprising an effective amount of an inhibitor of TC1.

14. A method for detecting a tumor in a subject, comprising detecting the presence of tumor marker protein TC1 in a sample of body fluid from said subject.

15. A method for detecting a in a subject comprising the steps of:

providing a sample of body fluid from said subject;

contacting said sample with a monoclonal antibody specific for an epitope of tumor marker protein TC1; and

detecting the presence of TC1 protein in said sample, wherein the presence of TC1 protein in said sample is indicative of the presence of a tumor in said subject.

16. The method of claim 14 or 15, wherein said body fluid is selected from the group consisting of blood, urine and sputum.

17. A method for detecting a tumor in a subject, comprising detecting the presence of tumor marker protein TC1, or of mRNA encoding tumor marker protein TC1, in a sample of a tissue section from said subject.

18. A method for detecting an invasive or metastatic tumor comprising the steps of:

providing a sample of a formalin-fixed or paraffin-embedded tissue section from said subject;

contacting said sample with a monoclonal antibody specific for an epitope of tumor marker protein TC1 in formalin-fixed or paraffin-embedded tissue sections; and

determining the level of TC1 protein in said sample, wherein the level of TC1 protein in said sample is related to the presence of an invasive or metastatic tumor in said subject.

19. The method of claim 17 or 18, wherein said tissue is breast, colon, or gastrointestinal tract tissue.

20. The method of claim 18, wherein said monoclonal antibody is a monoclonal antibody produced by the hybridoma cell line ATCC No. HB 11481.

21. The method of claim 18, wherein said monoclonal antibody is a monoclonal antibody that binds to the same antigenic determinant as a monoclonal antibody produced by the hybridoma cell line ATCC No. HB 11481.

22. A kit for diagnosis of an invasive or metastatic tumor in a subject, comprising

the monoclonal antibody of claim 1 or claim 2.

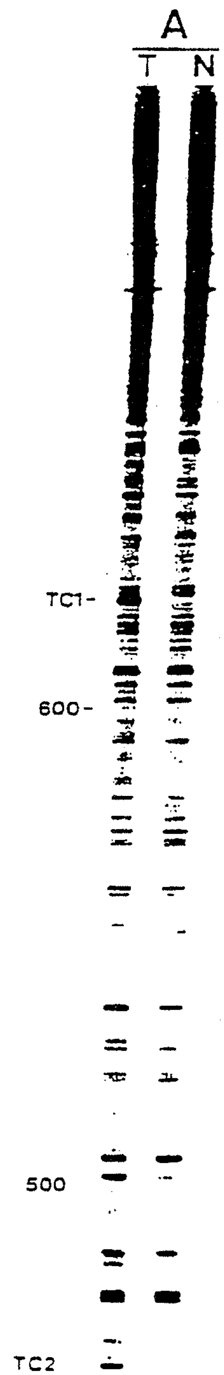


Fig. 1

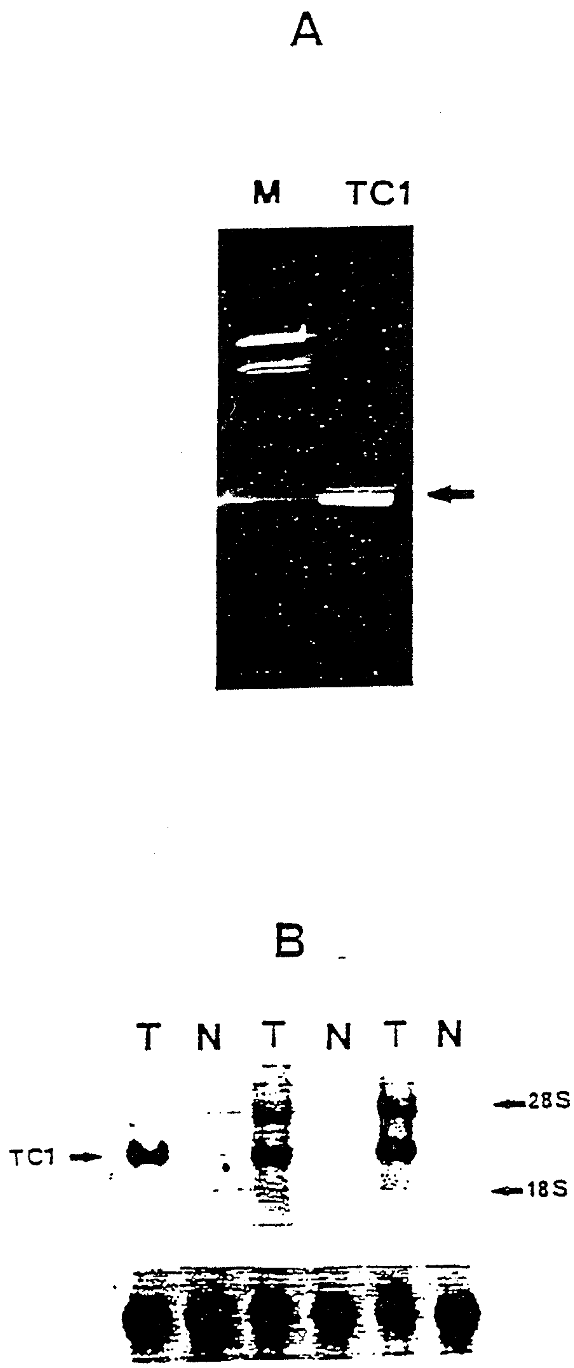


Fig. 2

```
1/1                                     31/11
CTG ATC CAT GGG AAC CAG ATT GCA ACA AAT GGT GTT GTC CAT GTC ATT GAC CGT GTG CTT
 L   I   H   G   N   Q   I   A   T   N   G   V   V   H   V   I   D   R   V   L
61/21                                   91/31
ACA CAA ATT GGT ACC TCA ATT CAA GAC TTC ATT GAA GCA GAA GAT GAC CTT TCA TCT TTT
 T   Q   I   G   T   S   I   Q   D   F   I   E   A   E   D   D   L   S   S   F
121/41                                  151/51
AGA GCA GCT GCC ATC ACA TCG GAC ATA TTG GAG GCC CTT GGA AGA GAC GGT CAC TTC ACA
 R   A   A   A   I   T   S   D   I   L   E   A   L   G   R   D   G   H   F   T
181/61                                  211/71
CTC TTT GCT CCC ACC AAT GAG GCT TTT GAG AAA CTT CCA CGA GGT GTC CTA GAA AGG ATC
 L   F   A   P   T   N   E   A   F   E   K   L   P   R   G   V   L   E   R   I
241/81                                  271/91
ATG GGA GAC AAA GTG GCT TCC GAA GCT CTT ATG AAG TAC CAC ATC TTA AAT ACT CTC CAG
 M   G   D   K   V   A   S   E   A   L   M   K   Y   H   I   L   N   T   L   Q
301/101                                 331/111
TGT TCT GAG TCT ATT ATG GGA GGA GCA GTC TTT GAG ACG CTG GAA GGA AAT ACA ATT GAG
 C   S   E   S   I   M   G   G   A   V   F   E   T   L   E   G   N   T   I   E
361/121                                 391/131
ATA GGA TGT GAC GGT GAC AGT ATA ACA GTA AAT GGA ATC AAA ATG GTG AAC AAA AAG GAT
 I   G   C   D   G   D   S   I   T   V   N   G   I   K   M   V   N   K   K   D
421/141                                 451/151
ATT GTG ACA AAT AAT GGT GTG ATC CAT TTG ATT GAT CAG GTC CTA ATT CCT GAT TCT GCC
 I   V   T   N   N   G   V   I   H   L   I   D   Q   V   L   I   P   D   S   A
481/161                                 511/171
AAA CAA GTT ATT GAG CTG GCT GGA AAA CAG CAA ACC ACC TTC ACG GAT CTT GTG GCC CAA
 K   Q   V   I   E   L   A   G   K   Q   Q   T   T   F   T   D   L   V   A   Q
541/181                                 571/191
TTA GGC TTG GCA TCT GCT CTG AGG CCA GAT GGA GAA TAC ACT TTG CTG GCA CCT GTG AAT
 L   G   L   A   S   A   L   R   P   D   G   E   Y   T   L   L   A   P   V   N
601/201                                 631/211
AAT GCA TTT TCT GAT GAT ACT CTC AGC ATG GAT CAG
 N   A   F   S   D   D   T   L   S   M   D   Q
```

Fig. 3

TCI-DNA -> Genes

DNA sequence 3126 b.p. gccaccatgtag ... ctttctcgtgcc linear

Fig. 4

1 gccaccatgtagcccgcgtcaccgttctgcgcattccgcagc ATG GCT CTG CCT GCC CGA ATC CTC GCT

1 M A L P A R I L A

70 CTG GCC CTC GCA CTG GCG CTC GGA CCC GCC GTG ACA CTG GCC AAC CCG GCG AGA ACG CCG

10 L A L A L A L G P A V T L A N P A R T P

130 TAC GAG CTG GTA CTC CAG AAG AGC TCG GCA CGA GGG GGT CGG GAC CAA GGC CCA AAT GTC

30 Y E L V L Q K S S A R G G R D Q G P N V

190 TGT GCC CTT CAA CAG ATT TTG GGC ACC AAA AAG AAA TAC TTC AGC ACT TGT AAG AAC TGG

50 C A L Q Q I L G T K K K Y F S T C K N W

250 TAT AAA AAG TCC ATC TGT GGA CAG AAA ACG ACT GTG TTA TAT GAA TGT TGC CCT GGT TAT

70 Y K K S I C G Q K T T V L Y E C C P G Y

310 ATG AGA ATG GAA GGA ATG AAA GGC TGC CCA GCA GTT TTG CCC ATT GAC CAT GTT TAT GGC

90 M R M E G M K G C P A V L P I D H V Y G

370 ACT CTG GGC ATC GTG GGA GCC ACC ACA ACG CAG CGC TAT TCT GAC GCC TCA AAA CTG AGG

110 T L G I V G A T T T Q R Y S D A S K L R

430 GAG GAG ATC GAG GGA AAG GGA TCC TTC ACT TAC TTT GCA CCG AGT AAT GAG GCT TGG GAC

130 E E I E G K G S F T Y F A P S N E A W D

490 AAC TTG GAT TCT GAT ATC CGT AGA GGT TTG GAG AGC AAC GTG AAT GTT GAA TTA CTG AAT

150 N L D S D I R R G L E S N V N V E L L N

550 GCT TTA CAT AGT CAC ATG ATT AAT AAG AGA ATG TTG ACC AAG GAC TTA AAA AAT GGC ATG

170 A L H S H M I N K R M L T K D L K N G M

610 ATT ATT CCT TCA ATG TAT AAC AAT TTG GGG CTT TTC ATT AAC CAT TAT CCT AAT GGG GTT

190 I I P S M Y N N L G L F I N H Y P N G V

670 GTC ACT GTT AAT TGT GCT CGA ATC ATC CAT GGG AAC CAG ATT GCA ACA AAT GGT GTT GTC

210 V T V N C A R I I H G N Q I A T N G V V

730 CAT GTC ATT GAC CGT GTG CTT ACA CAA ATT GGT ACC TCA ATT CAA GAC TTC ATT GAA GCA

230 H V I D R V L T Q I G T S I Q D F I E A

790 GAA GAT GAC CTT TCA TCT TTT AGA GCA GCT GCC ATC ACA TCG GAC ATA TTG GAG GCC CTT

250 E D D L S S F R A A A I T S D I L E A L

850 GGA AGA GAC GGT CAC TTC ACA CTC TTT GCT CCC ACC AAT GAG GCT TTT GAG AAA CTT CCA

270 G R D G H F T L F A P T N E A F E K L P

910 CGA GGT GTC CTA GAA AGG ATC ATG GGA GAC AAA GTG GCT TCC GAA GCT CTT ATG AAG TAC

290 R G V L E R I M G D K V A S E A L M K Y

970 CAC ATC TTA AAT ACT CTC CAG TGT TCT GAG TCT ATT ATG GGA GGA GCA GTC TTT GAG ACG

310 H I L N T L Q C S E S I M G G A V F E T

1030 CTG GAA GGA AAT ACA ATT GAG ATA GGA TGT GAC GGT GAC AGT ATA ACA GTA AAT GGA ATC

330 L E G N T I E I G C D G D S I T V N G I

1090 AAA ATG GTG AAC AAA AAG GAT ATT GTG ACA AAT AAT GGT GTG ATC CAT TTG ATT GAT CAG

350 K M V N K K D I V T N N G V I H L I D Q

1150 GTC CTA ATT CCT GAT TCT GCC AAA CAA GTT ATT GAG CTG GCT GGA AAA CAG CAA ACC ACC

370 V L I P D S A K Q V I E L A G K Q Q T T

1210 TTC ACG GAT CTT GTG GCC CAA TTA GGC TTG GCA TCT GCT CTG AGG CCA GAT GGA GAA TAC

390 F T D L V A Q L G L A S A L R P D G E Y

1270 ACT TTG CTG GCA CCT GTG AAT AAT GCA TTT TCT GAT GAT ACT CTC AGC ATG GAT CAG CGC

410 T L L A P V N N A F S D D T L S M D Q R

1330 CTC CTT AAA TTA ATT CTG CAG AAT CAC ATA TTG AAA GTA AAA GTT GGC CTT AAT GAG CTT

430 L L K L I L Q N H I L K V K V G L N E L

1390 TAC AAC GGG CAA ATA CTG GAA ACC ATC GGA GGC AAA CAG CTC AGA GTC TTC GTA TAT CGT

450 Y N G Q I L E T I G G K Q L R V F V Y R

1450 ACA GCT GTC TGC ATT GAA AAT TCA TGC ATG GAG AAA GGG AGT AAG CAA GGG AGA AAC GGT

470 T A V C I E N S C M E K G S K Q G R N G

1510 GCG ATT CAC ATA TTC CGC GAG ATC ATC AAG CCA GCA GAG AAA TCC CTC CAT GAA AAG TTA

490 A I H I F R E I I K P A E K S L H E K L

1570 AAA CAA GAT AAG CGC TTT ACG ACC TTC CTC AGC CTA CTT GAA GCT GCA GAC TTG AAA GAG

510 K Q D K R F T T F L S L L E A A D L K E

1630 CTC CTG ACA CAA CCT GGA GAC TGG ACA TTA TTT GTG CCA ACC AAT GAT GCT TTT AAG GGA

530 L L T Q P G D W T L F V P T N D A F K G

1690 ATG ACT AGT GAA GAA AAA GAA ATT CTG ATA CGG GAC AAA AAT GCT CTT CAA AAC ATC ATT

550 M T S E E K E I L I R D K N A L Q N I I

1750 CTT TAT CAC CTG ACA CCA GGA GTT TTC ATT GGA AAA GGA TTT GAA CCT GGT GTT ACT AAC

570 L Y H L T P G V F I G K G F E P G V T N

1810 ATT TTA AAG ACC ACA CAA GGA AGC AAA ATC TTT CTG AAA GAA GTA AAT GAT ACA CTT CTG

590 I L K T T Q G S K I F L K E V $\boxed{N\ D\ T}$ L L

1870 GTG AAT GAA TTG AAA TCA AAA GAA TCT GAC ATC ATG ACA ACA AAT GGT GTA ATT CAT GTT

610 V N E L K S K E S D I M T T N G V I H V

1930 GTA GAT AAA CTC CTC TAT CCA GCA GAC ACA CCT GTT GGA AAT GAT CAA CTG CTG GAA ATA

630 V D K L L Y P A D T P V G N D Q L L E I

1990 CTT AAT AAA TTA ATC AAA TAC ATC CAA ATT AAG TTT GTT CGT GGT AGC ACC TTC AAA GAA

650 L N K L I K Y I Q I K F V R G S T F K E

2050 ATC CCC GTG ACT GTC TAT AGA CCC ACA CTA ACA AAA GTC AAA ATT GAA GGT GAA CCT GAA

670 I P V T V Y R P T L T K V K I E G E P E

2110 TTC AGA CTG ATT AAA GAA GGT GAA ACA ATA ACT GAA GTG ATC CAT GGA GAG CCA ATT ATT

690 F R L I K E G E T I T E V I H G E P I I

2170 AAA AAA TAC ACC AAA ATC ATT GAT GGA GTG CCT GTG GAA ATA ACT GAA AAA GAG ACA CGA

710 K K Y T K I I D G V P V E I T E K E T R

2230 GAA GAA CGA ATC ATT ACA GGT CCT GAA ATA AAA TAC ACT AGG ATT TCT ACT GGA GGT GGA

730 E E R I I T G P E I K Y T R I S T G G G

2290 GAA ACA GAA GAA ACT CTG AAG AAA TTG TTA CAA GAA GAC ACA CCC GTG AGG AAG TTG CAA

750 E T E E T L K K L L Q E D T P V R K L Q

2350 GCC AAC AAA AAA AGT TCA AGG ATC TAG aagacgattaagggaaggtcgttctcagtgaaaatccaa

770 A N K K S S R I *

2421 agaaaaaatgtttatacaaccctaagtcaataacctgaccttagaaaattgtgagagccaagttgacttcag

2501 aacatcagcacaaagaagcaatcatcaaataattctgaacacaaatttaatattttttttctgaatgagaaacatg

2581 gaaattgtggagttagcctcctgtggtaaaggaattgaagaaaatataacaccttacacccttttcatcttgacatt

2661 aagttctggctaactttggaatccattagagaaaaatccttgtcaccagattcattacaattcaaatcgaagagttg

2741 actgttatcccattgaaaagaccgagccttgtatgtatgttatggatacataaaatgcacgcaagccattatctctc

2821 gggaagctaagttataaaaataggtgcttggtgtacaaactttttatgatcaaaaggctttgcacatttctatatgagt

2901 gggtttactggtaaattatgttatttttacaactaattttgtactctcagaatgtttgtcatatgcttcttgcaatgca

2981 tatttttaatctcaaacgtttc$\boxed{AATAAA}$accattttcagatataaagagaattacttcaaattgagtaattcagaaa

3061 actcaagatttaagttaaaaagtggtttggacttgggaataggactttatacctctttctcgtgcc

Fig. 5

Four Repeats of TC1 Protein

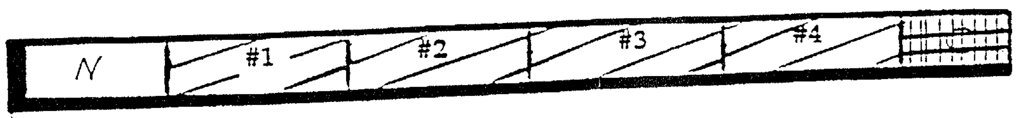
```
          aa#
TC1 #2   276  T L F A P T N E A F . E K L P R G V L E R I M . . G D K V A S E A L . . . M K Y H I L H
TC1 #4   538  T L F V P T N D A F . . K G N T S . E E K E I L I R D K N A . . . L Q N I I L Y H L . .
TC1 #1   139  T Y F A P S H E A W . . D N L D S . D I R R G L . E S N V . N V E L L N A L H S H N I H
TC1 #3   411  T L L A P V H H A F S D D T L . S M D Q . R L L . K L T L Q N H I L . . . . V K V G L H

TC1 #2        T L Q C S . . . E S I N G G A V F E T L E . . . G N T I E I . G C D G D S I T V H . G I .
TC1 #4        T P G V F I G . K G F E P G . V T N I L K T T Q G S K I F L . K E V H D T L L V H . E L .
TC1 #1        K R M L T K D L K N . . . G M I I P S H Y N N L G . . L F I N H Y P N G V V T V H . C A .
TC1 #3        E L Y N G Q I L E T I . G G . . . . . . K . Q L R . . V F V . . Y R T A V C I E H S C H E

TC1 #2        K M V H K K . D I V T N H G V I H L I D Q V L I . P D S . . A K Q V . I E L A . G K Q Q
TC1 #4        K S   . K E S D I M T T H G V I H V V D K I L Y . P A D T P V G N D . Q L L E . I L N K
TC1 #1        R I I H G N . Q I A T . H G V V H V I D R V L T . Q I G T S I . Q D F I E A E D D L . .
TC1 #3        K G S . K . . Q G R . . H G A I H . I F R E T I K P A E K S Z H E . . K L K Q . D K R F

TC1 #2        T T F T D L V A Q . L G . . . . . L A S A L . R P D G E Y                           410
TC1 #4        L I K Y I Q I K F V R G S T F K E I P V T V Y R P T L T K                           671
TC1 #1        S S F . R A A A I T S D I L E . C . . . A L G R . D G H F                           275
TC1 #3        T T F L S L E A A D L . . . . K E L . . . L T Q P . G D W                           517
```

A



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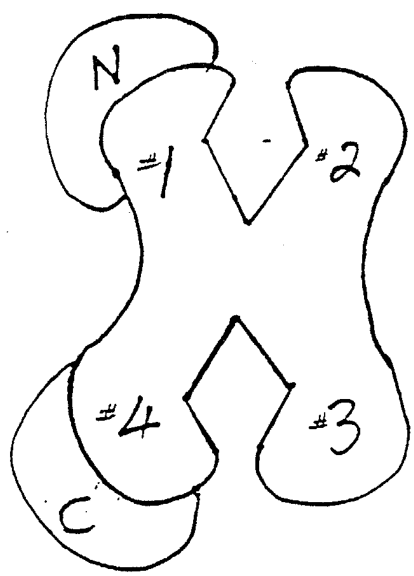
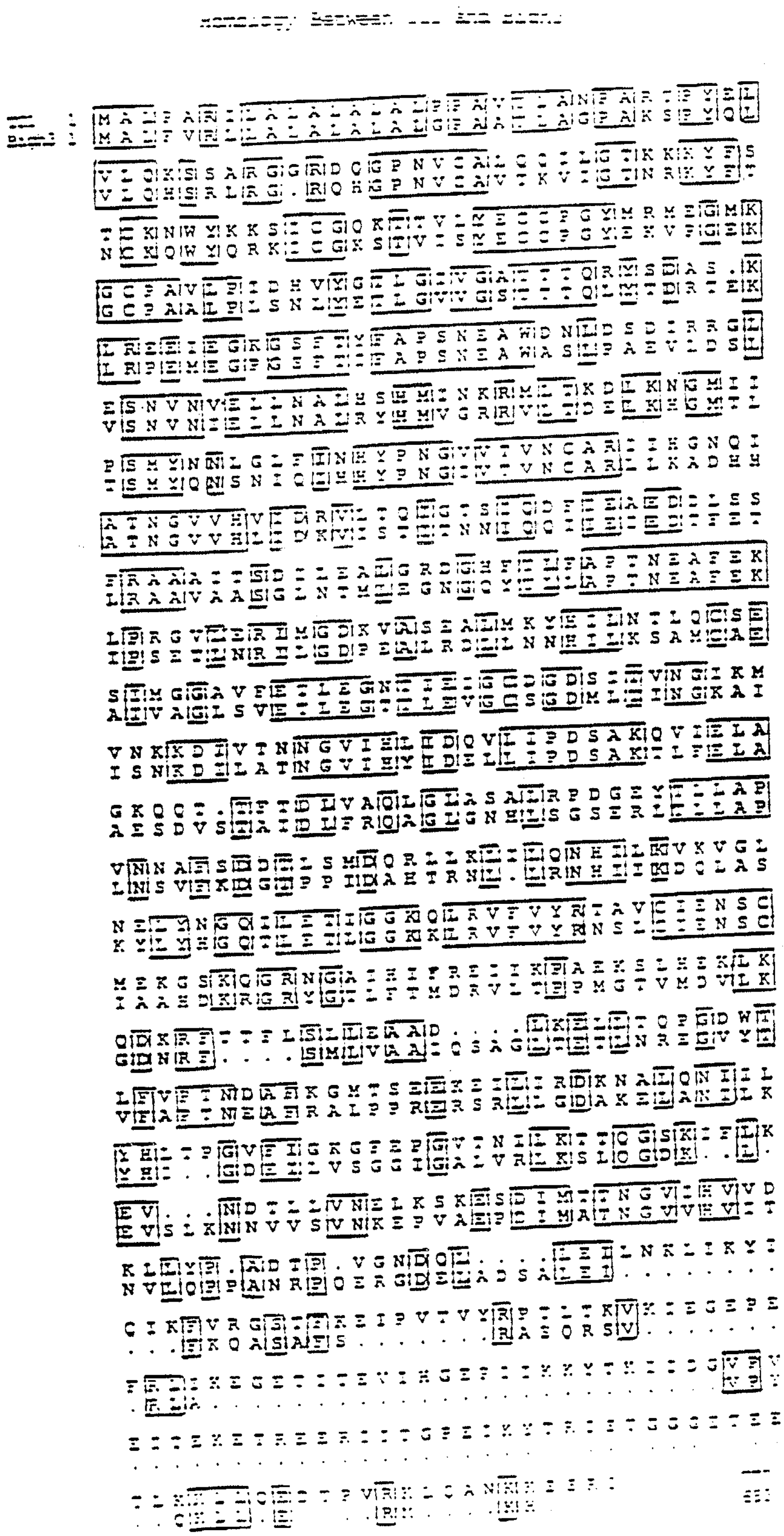


Fig. 6

Fig. 7



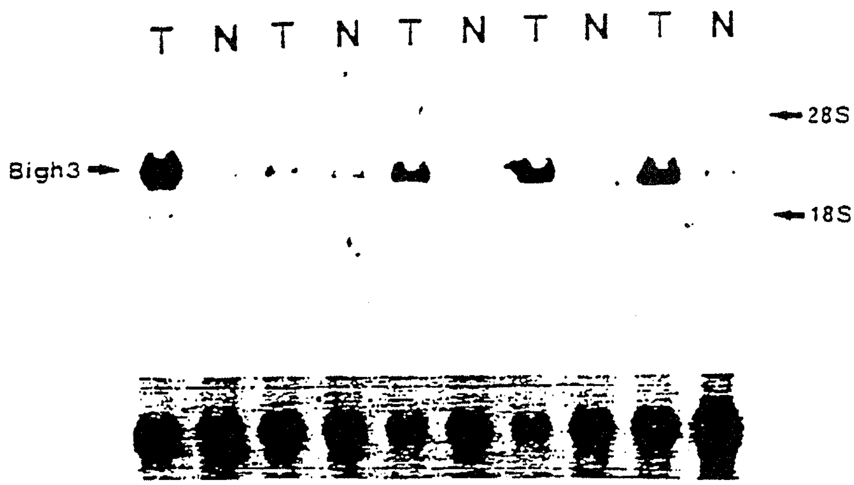


Fig. 8

Homology Between TCl and Fasciclin I

```
TCl 503 A E K S L H E K L K Q D K R F T T F L S L L E A A D L
GrF  26 G E K S L E Y K I R D D P D L S Q F Y S W L E H N E V
DrF  19 A A A D L A D K L R D D S E L S Q F Y S L L E S N Q I

TCl     K E L L T Q P G D W T L F V P T N D A F . . . K G M T
GrF     A N S T L Q L R Q V T V F A P T N L A F Q N Y K A R D
DrF     A N S T L S L R S C T I F V P T N E A F Q R Y K . . .

TCl     S E E K E I L I R D K N A L Q N I I L Y H L T P G V F
GrF     G D E N I I L Y H M T N L A H S . . L D Q L G H K V L
DrF     S K T A H V L Y H I T T E A Y T . . Q K R L P N T V S

TCl     I G K . G E E P G V T N I L K T T Q G . S K I F L K E
GrF     S E L D G N P P . . . . . L W I T R R R D T I F . . .
DrF     S D M A G N P P . . L Y I T K N S N G . . D I F . . .

TCl     V N D . T L L V N E L K S K E S D I M . T T N G V I H
GrF     V N N A R V L T . E R S N Y E A V N R H G K K Q V L H
DrF     V G N A R I I P . S L S V . E T N S D . G K R Q I M H

TCl     V V D K L L Y P A D . T P . V G . . . N D Q L L E I L
GrF     V V D S V L E P V W S T S . . G Q L Y N P D A F Q F L
DrF     I I D E V L E P L . . T V K A G H S D T P N N P . . .

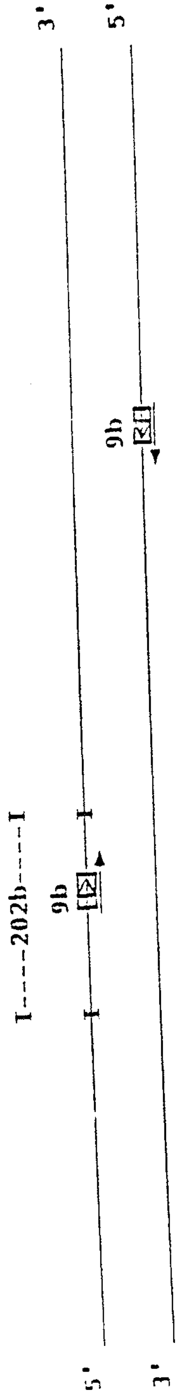
TCl     N K I I K Y I Q I K F V R G S T F K E I P V I V Y R P
GrF     N Q . S E N L D L G L H R V R S F R Q R . V F Q . N Q
DrF     N A I . K F . . L K N A E E F N V D N I G V R T Y R S

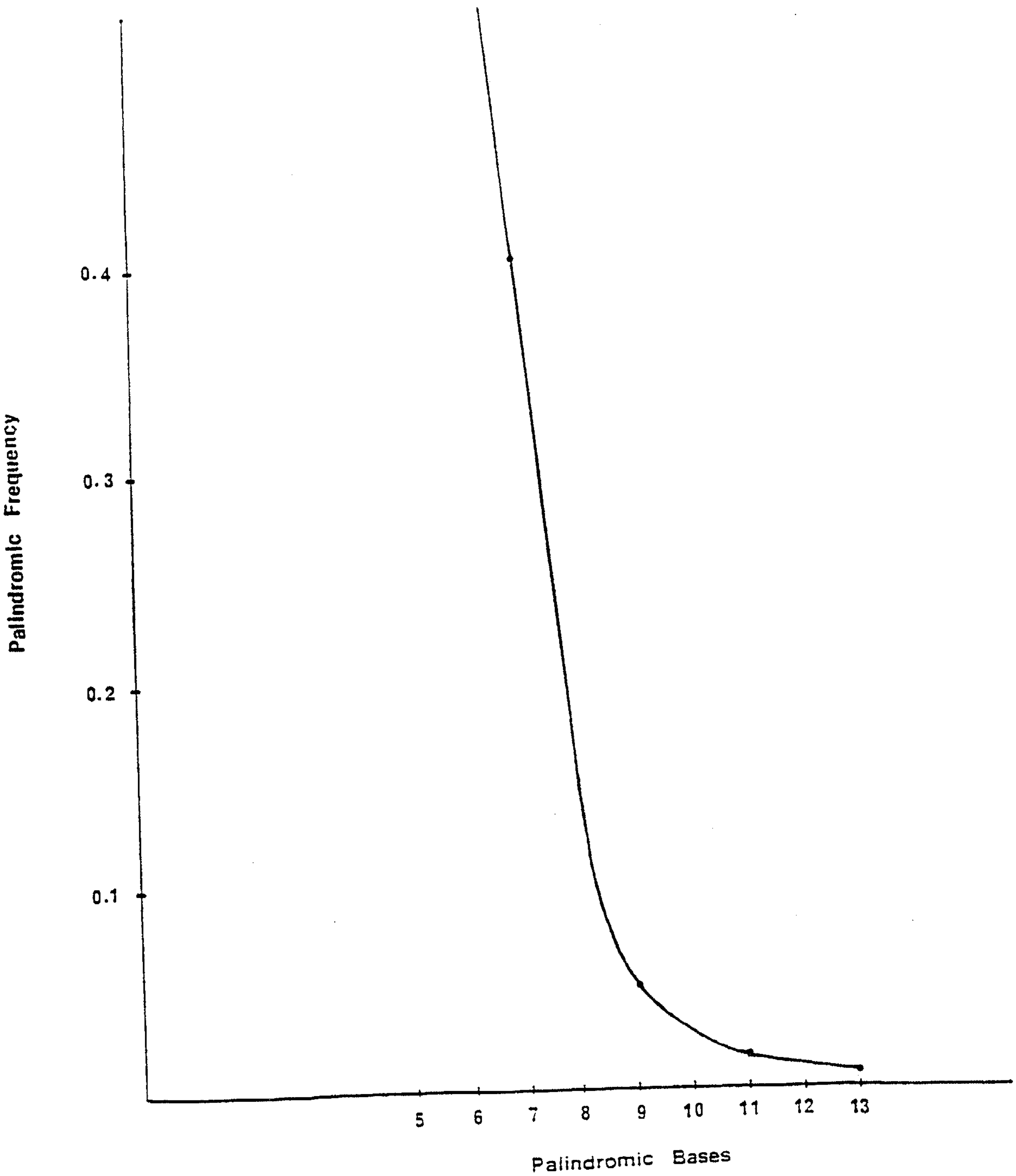
TCl     T L T K V K I E G E P E F R L I K E G E T I T E V I H
GrF     K Q N D F K L E G K H T E . F I P V D E G F K P L P R
DrF     Q V T M A K K E S V Y D A A G Q H T F L V P V D E G F

TCl     G E P I I K K                                     706
GrF     P E K I D Q K                                     221
DrF     K I S A R S S                                     211
```

Fig. 9

Fig. 10





Palindromic Frequency
0.4
0.3
0.2
0.1
5
6
7
8
9
10
11
12
13
Palindromic Bases

Fig. 11

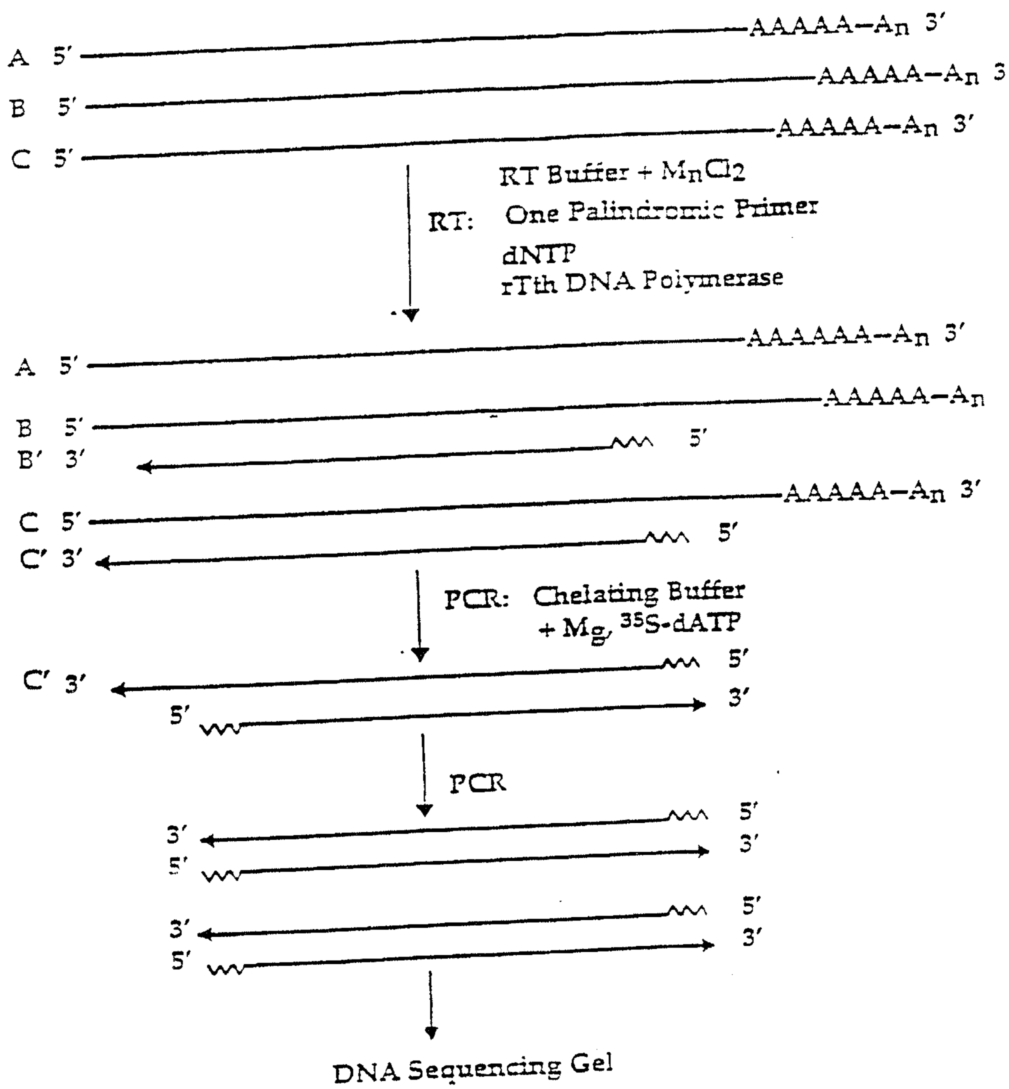

A 5' AAAAA–An 3'
B 5' AAAAA–An 3'
C 5' AAAAA–An 3'
RT Buffer + MnCl2
RT: One Palindromic Primer
dNTP
rTth DNA Polymerase
A 5' AAAAAA–An 3'
B 5' AAAAA–An
B' 3' 5'
C 5' AAAAA–An 3'
C' 3' 5'
PCR: Chelating Buffer
+ Mg, 35S-dATP
C' 3' 5'
5' 3'
PCR
3' 5'
5' 3'
3' 5'
5' 3'
DNA Sequencing Gel

Fig. 12

Fig. 13

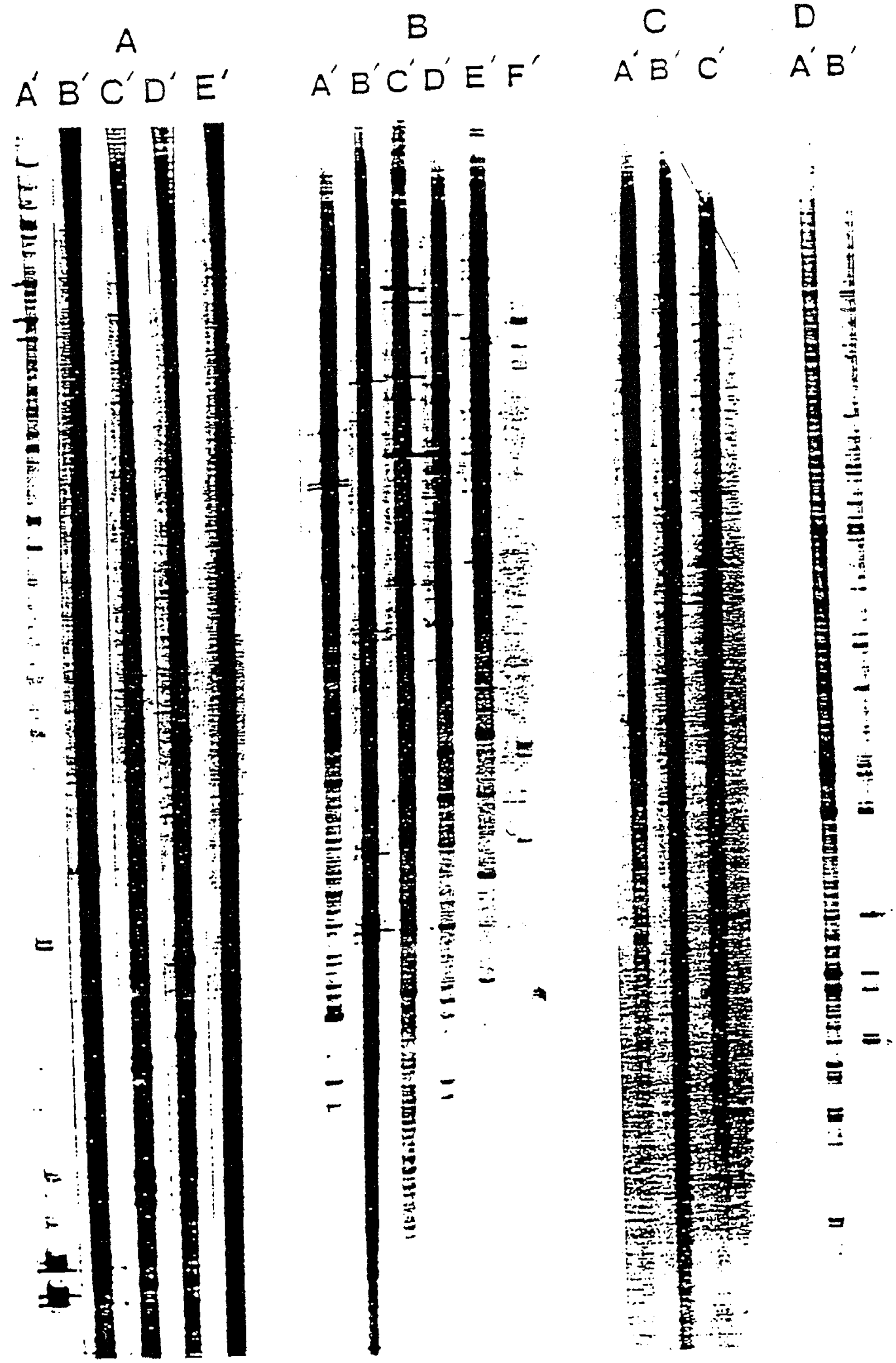
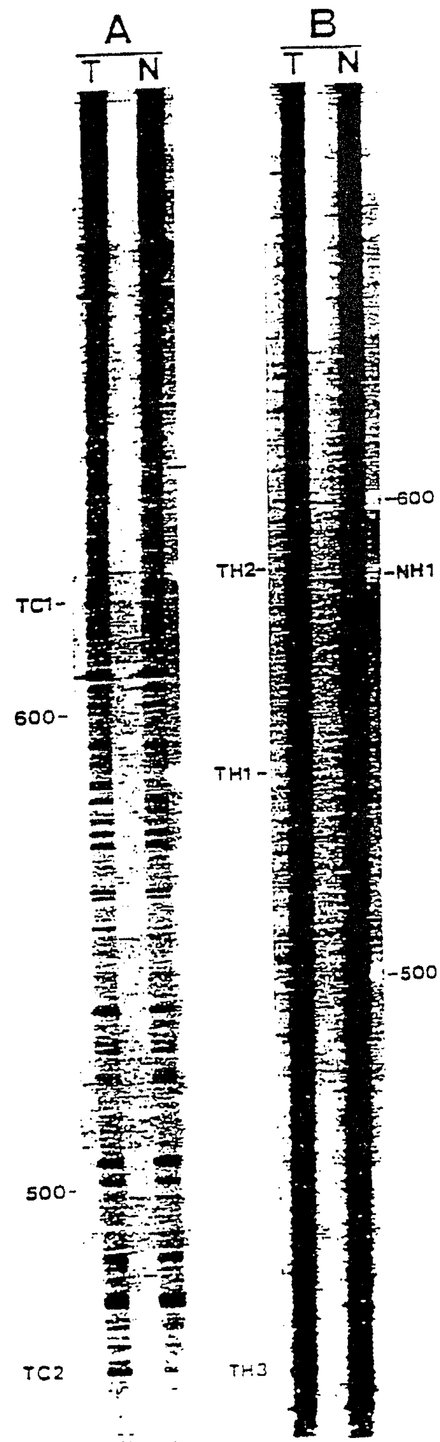


Fig. 14



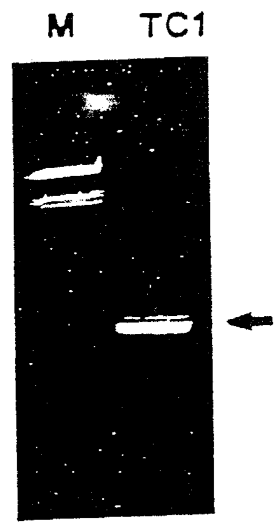


Fig. 15

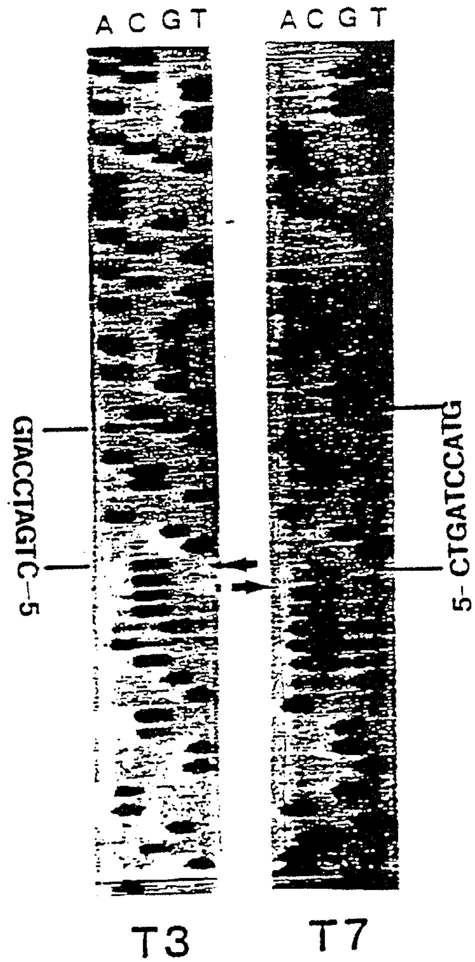
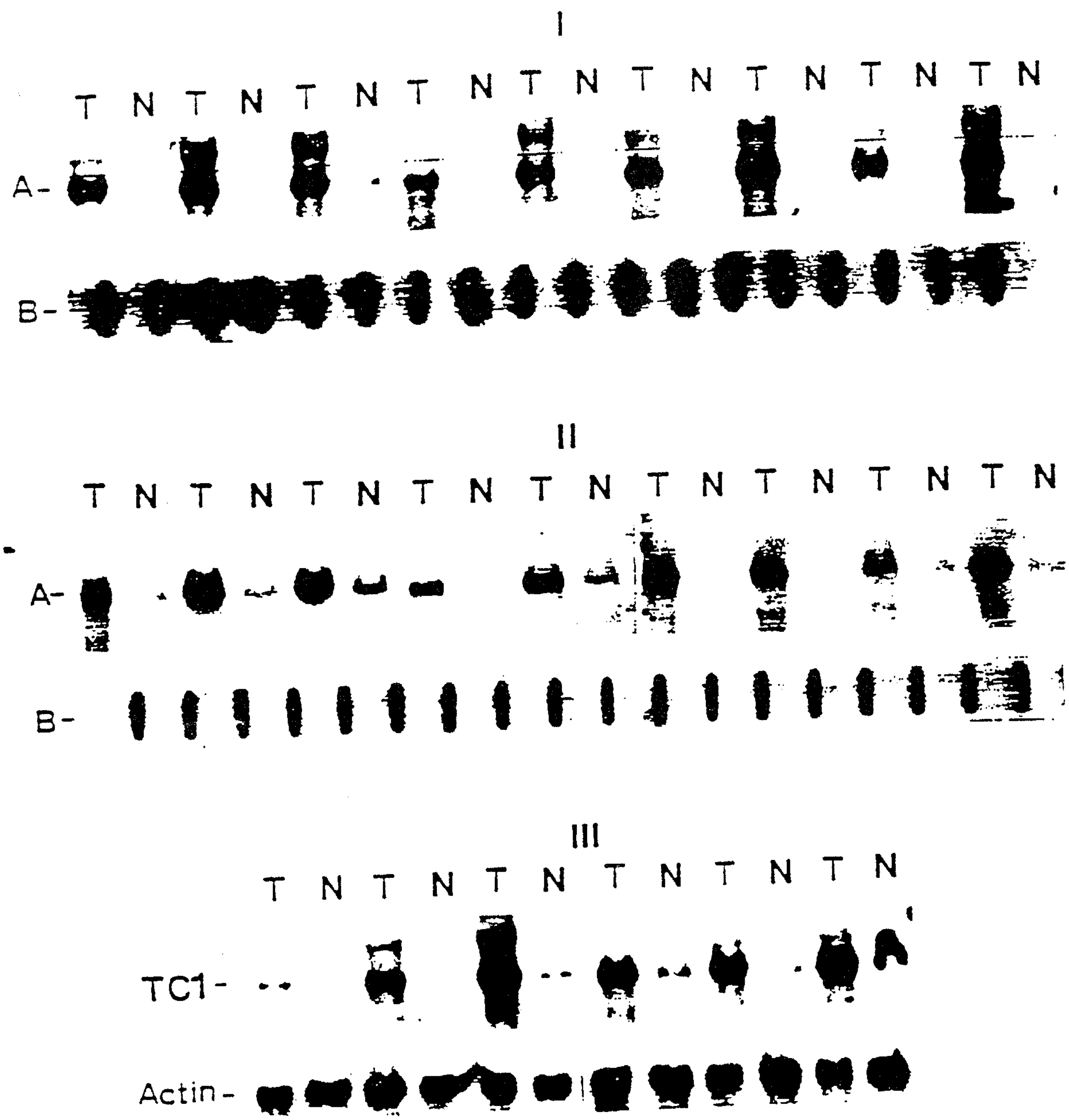


Fig. 16

Fig. 17


I
T N T N T N T N T N T N T N T N T N
A-
B-
II
T N T N T N T N T N T N T N T N T N
A-
B-
III
T N T N T N T N T N T N
TC1-
Actin-

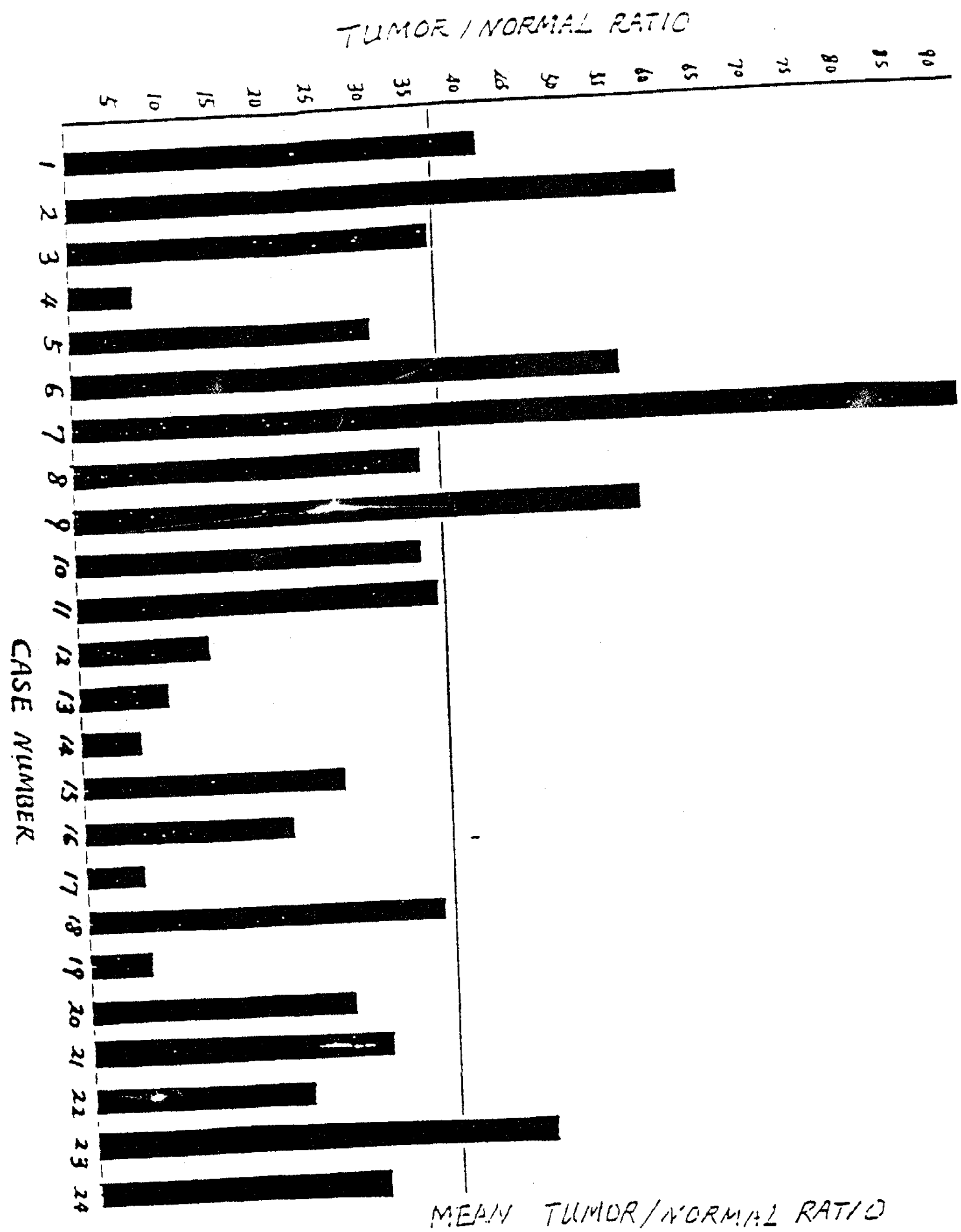

TUMOR / NORMAL RATIO
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
CASE NUMBER
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
MEAN TUMOR/NORMAL RATIO

Fig. 18

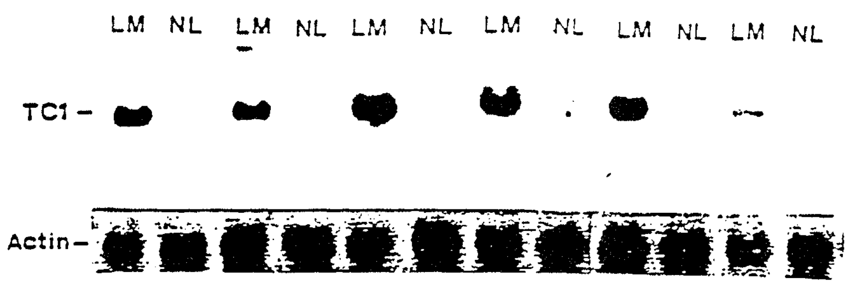
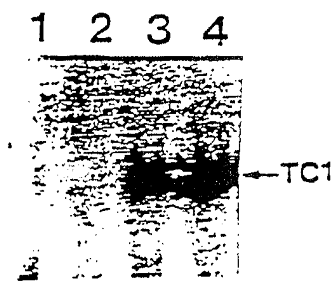


Fig. 19



1, HT29 CELL 3, HT29 TUMOR
2, CX–1 CELL 4, CX–1 TUMOR

Fig. 20

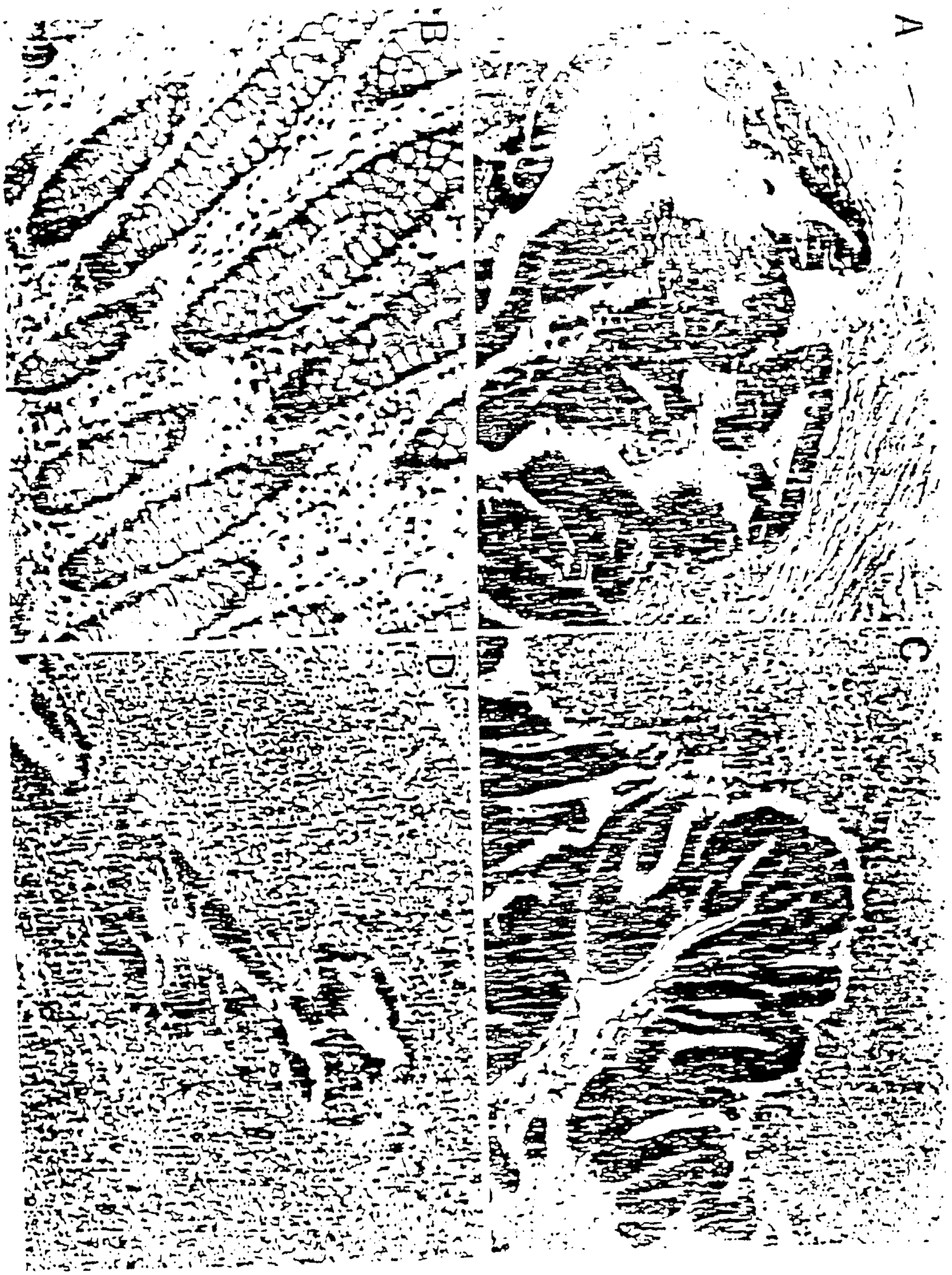


Fig. 21

Fig. 22

Fig. 23

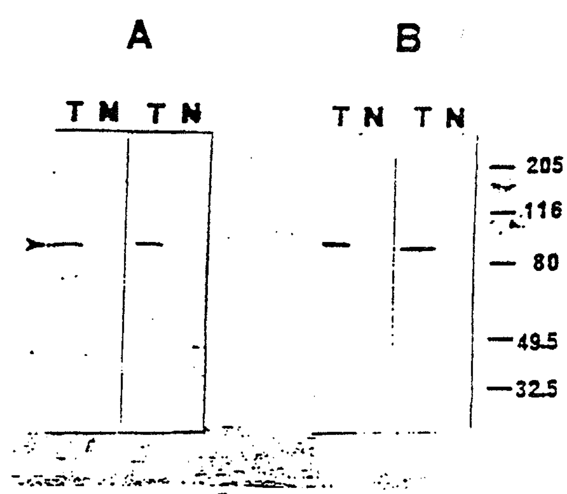
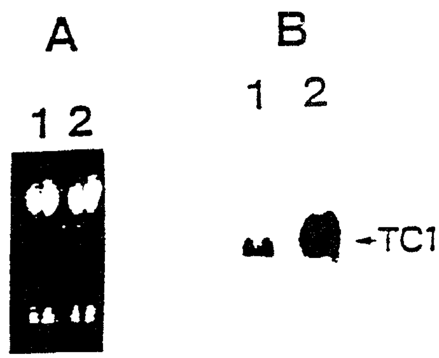


Fig. 24



1, JMN ; 2, JMN1B

Fig. 25

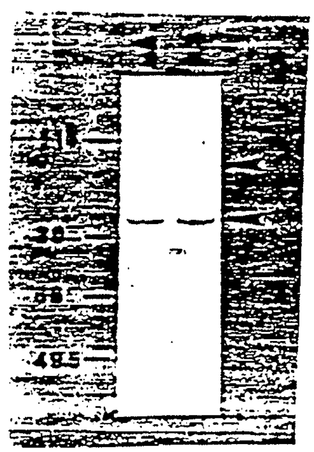


Fig. 26

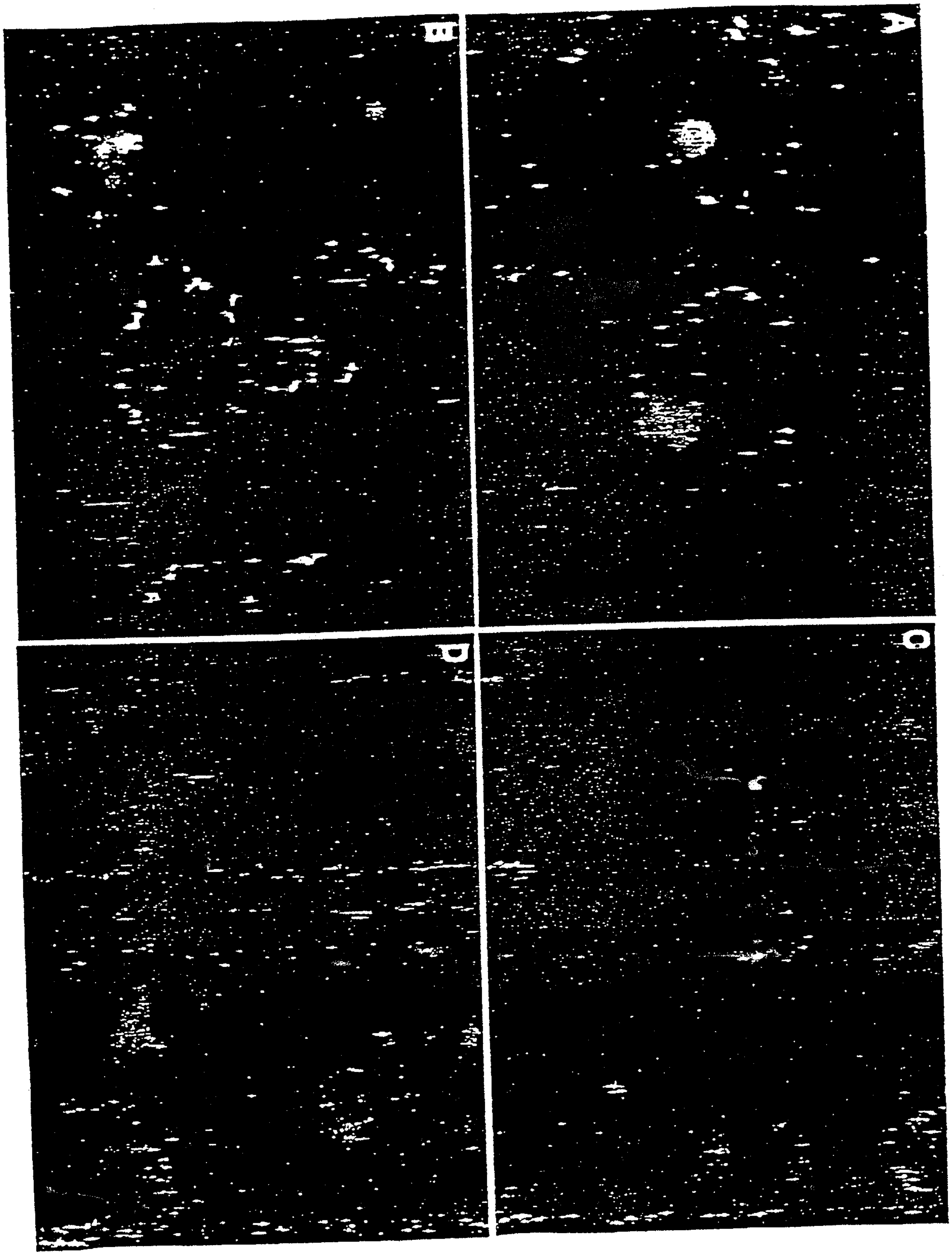


Fig. 27

Nucleotide Sequence and Deduced Amino Acid Sequence of TC1

```
  1 attcgacgtctcgtccacaacagaactgcaacggagagactcaag ATG ATT CCC TTT TTA CCC ATG TTT   69
  1                                               M   I   P   F   L   P   M   F      8

 70 TCT CTA CTA TTG CTG CTT ATT GTT AAC CCT ATA AAC GCC AAC AAT CAT TAT GAC AAG ATC  129
  9 S   L   L   L   L   L   I   V   N   P   I   N   A   N   N   H   Y   D   K   I     28

130 TTG GCT CAT AGT CGT ATC AGG GGT CGG GAC CAA GGC CCA AAT GTC TGT GCC CTT CAA CAG  189
 29 L   A   H   S   R   I   R   G   R   D   Q   G   P   N   V   C   A   L   Q   Q     48

190 ATT TTG GGC ACC AAA AAG AAA TAC TTC AGC ACT TGT AAG AAC TGG TAT AAA AAG TCC ATC  249
 49 I   L   G   T   K   K   K   Y   F   S   T   C   K   N   W   Y   K   K   S   I     68

250 TGT GGA CAG AAA ACG ACT GTG TTA TAT GAA TGT TGC CCT GGT TAT ATG AGA ATG GAA GGA  309
 69 C   G   Q   K   T   T   V   L   Y   E   C   C   P   G   Y   M   R   M   E   G     88

310 ATG AAA GGC TGC CCA GCA GTT TTG CCC ATT GAC CAT GTT TAT GGC ACT CTG GGC ATC GTG  369
 89 M   K   G   C   P   A   V   L   P   I   D   H   V   Y   G   T   L   G   I   V    108

370 GGA GCC ACC ACA ACG CAG CGC TAT TCT GAC GCC TCA AAA CTG AGG GAG GAG ATC GAG GGA  429
109 G   A   T   T   T   Q   R   Y   S   D   A   S   K   L   R   E   E   I   E   G    128

430 AAG GGA TCC TTC ACT TAC TTT GCA CCG AGT AAT GAG GCT TGG GAC AAC TTG GAT TCT GAT  489
129 K   G   S   F   T   Y   F   A   P   S   N   E   A   W   D   N   L   D   S   D    148

490 ATC CGT AGA GGT TTG GAG AGC AAC GTG AAT GTT GAA TTA CTG AAT GCT TTA CAT AGT CAC  549
149 I   R   R   G   L   E   S   N   V   N   V   E   L   L   N   A   L   H   S   H    168

550 ATG ATT AAT AAG AGA ATG TTG ACC AAG GAC TTA AAA AAT GGC ATG ATT ATT CCT TCA ATG  609
169 M   I   N   K   R   M   L   T   K   D   L   K   N   G   M   I   I   P   S   M    188

610 TAT AAC AAT TTG GGG CTT TTC ATT AAC CAT TAT CCT AAT GGG GTT GTC ACT GTT AAT TGT  669
189 Y   N   N   L   G   L   F   I   N   H   Y   P   N   G   V   V   T   V   N   C    208
```

FIG. 28A

```
670 GCT CGA ATC ATC CAT GGG AAC CAG ATT GCA ACA AAT GGT GTT GTC CAT GTC ATT GAC CGT  729
209 A   R   I   I   H   G   N   Q   I   A   T   N   G   V   V   H   V   I   D   R    228

730 GTG CTT ACA CAA ATT GGT ACC TCA ATT CAA GAC TTC ATT GAA GCA GAA GAT GAC CTT TCA  789
229 V   L   T   Q   I   G   T   S   I   Q   D   F   I   E   A   E   D   D   L   S    248

790 TCT TTT AGA GCA GCT GCC ATC ACA TCG GAC ATA TTG GAG GCC CTT GGA AGA GAC GGT CAC  849
249 S   F   R   A   A   A   I   T   S   D   I   L   E   A   L   G   R   D   G   H    268

850 TTC ACA CTC TTT GC' CCC ACC AAT GAG GCT TTT GAG AAA CTT CCA CGA GGT GTC CTA GAA  909
269 F   T   L   F   A   P   T   N   E   A   F   E   K   L   P   R   G   V   L   E    288

910 AGG ATC ATG GGA GAC AAA GTG GCT TCC GAA GCT CTT ATG AAG TAC CAC ATC TTA AAT ACT  969
289 R   I   M   G   D   K   V   A   S   E   A   L   M   K   Y   H   I   L   N   T    308

970 CTC CAG TGT TCT GAG TCT ATT ATG GGA GGA GCA GTC TTT GAG ACG CTG GAA GGA AAT ACA  1029
309 L   Q   C   S   E   S   I   M   G   G   A   V   F   E   T   L   E   G   N   T    328

1030 ATT GAG ATA GGA TGT GAC GGT GAC AGT ATA ACA GTA AAT GGA ATC AAA ATG GTG AAC AAA  1089
329  I   E   I   G   C   D   G   D   S   I   T   V   N   G   I   K   M   V   N   K    348

1090 AAG GAT ATT GTG ACA AAT AAT GGT GTG ATC CAT TTG ATT GAT CAG GTC CTA ATT CCT GAT  1149
349  K   D   I   V   T   N   N   G   V   I   H   L   I   D   Q   V   L   I   P   D    368

1150 TCT GCC AAA CAA GTT ATT GAG CTG GCT GGA AAA CAG CAA ACC ACC TTC ACG GAT CTT GTG  1209
369  S   A   K   Q   V   I   E   L   A   G   K   Q   Q   T   T   F   T   D   L   V    388

1210 GCC CAA TTA GGC TTG GCA TCT GCT CTG AGG CCA GAT GGA GAA TAC ACT TTG CTG GCA CCT  1269
389  A   Q   L   G   L   A   S   A   L   R   P   D   G   E   Y   T   L   L   A   P    408

1270 GTG AAT AAT GCA TTT TCT GAT GAT ACT CTC AGC ATG GAT CAG CGC CTC CTT AAA TTA ATT  1329
409  V   N   N   A   F   S   D   D   T   L   S   M   D   Q   R   L   L   K   L   I    428

1330 CTG CAG AAT CAC ATA TTG AAA GTA AAA GTT GGC CTT AAT GAG CTT TAC AAC GGG CAA ATA  1389
429  L   Q   N   H   I   L   K   V   K   V   G   L   N   E   L   Y   N   G   Q   I    448
```

FIG. 28B

```
1390 CTG GAA ACC ATC GGA GGC AAA CAG CTC AGA GTC TTC GTA TAT CGT ACA GCT GTC TGC ATT 1449
 449 L   E   T   I   G   G   K   Q   L   R   V   F   V   Y   R   T   A   V   C   I   468

1450 GAA AAT TCA TGC ATG GAG AAA GGG AGT AAG CAA GGG AGA AAC GGT GCG ATT CAC ATA TTC 1509
 469 E   N   S   C   M   E   K   G   S   K   Q   G   R   N   G   A   I   H   I   F   488

1510 CGC GAG ATC ATC AAG CCA GCA GAG AAA TCC CTC CAT GAA AAG TTA AAA CAA GAT AAG CGC 1569
 489 R   E   I   I   K   P   A   E   K   S   L   H   E   K   L   K   Q   D   K   R   508

1570 TTT ACG ACC TTC CTC AGC CTA CTT GAA GCT GCA GAC TTG AAA GAG CTC CTG ACA CAA CCT 1629
 509 F   T   T   F   L   S   L   L   E   A   A   D   L   K   E   L   L   T   Q   P   528

1630 GGA GAC TGG ACA TTA TTT GTG CCA ACC AAT GAT GCT TTT AAG GGA ATG ACT AGT GAA GAA 1689
 529 G   D   W   T   L   F   V   P   T   N   D   A   F   K   G   M   T   S   E   E   548

1690 AAA GAA ATT CTG ATA CGG GAC AAA AAT GCT CTT CAA AAC ATC ATT CTT TAT CAC CTG ACA 1749
 549 K   E   I   L   I   R   D   K   N   A   L   Q   N   I   I   L   Y   H   L   T   568

1750 CCA GGA GTT TTC ATT GGA AAA GGA TTT GAA CCT GGT GTT ACT AAC ATT TTA AAG ACC ACA 1809
 569 P   G   V   F   I   G   K   G   F   E   P   G   V   T   N   I   L   K   T   T   588

1810 CAA GGA AGC AAA ATC TTT CTG AAA GAA GTA AAT GAT ACA CTT CTG GTG AAT GAA TTG AAA 1869
 589 Q   G   S   K   I   F   L   K   E   V   N   D   T   L   L   V   N   E   L   K   608

1870 TCA AAA GAA TCT GAC ATC ATG ACA ACA AAT GGT GTA ATT CAT GTT GTA GAT AAA CTC CTC 1929
 609 S   K   E   S   D   I   M   T   T   N   G   V   I   H   V   V   D   K   L   L   628

1930 TAT CCA GCA GAC ACA CCT GTT GGA AAT GAT CAA CTG CTG GAA ATA CTT AAT AAA TTA ATC 1989
 629 Y   P   A   D   T   P   V   G   N   D   Q   L   L   E   I   L   N   K   L   I   648

1990 AAA TAC ATC CAA ATT AAG TTT GTT CGT GGT AGC ACC TTC AAA GAA ATC CCC GTG ACT GTC 2049
 649 K   Y   I   Q   I   K   F   V   R   G   S   T   F   K   E   I   P   V   T   V   668

2050 TAT AGA CCC ACA CTA ACA AAA GTC AAA ATT GAA GGT GAA CCT GAA TTC AGA CTG ATT AAA 2109
 669 Y   R   P   T   L   T   K   V   K   I   E   G   E   P   E   F   R   L   I   K   688
```

FIG. 28C

```
2110 GAA GGT GAA ACA ATA ACT GAA GTG ATC CAT GGA GAG CCA ATT ATT AAA AAA TAC ACC AAA 2169
 689 E   G   E   T   I   T   E   V   I   H   G   E   P   I   I   K   K   Y   T   K    708

2170 ATC ATT GAT GGA GTG CCT GTG GAA ATA ACT GAA AAA GAG ACA CGA GAA GAA CGA ATC ATT 2229
 709 I   I   D   G   V   P   V   E   I   T   E   K   E   T   R   E   E   R   I   I    728

2230 ACA GGT CCT GAA ATA AAA TAC ACT AGG ATT TCT ACT GGA GGT GGA GAA ACA GAA GAA ACT 2289
 729 T   G   P   E   I   K   Y   T   R   I   S   T   G   G   G   E   T   E   E   T    748

2290 CTG AAG AAA TTG TTA CAA GAA GAG GTC ACC AAG GTC ACC AAA TTC ATT GAA GGT GGT GAT 2349
 749 L   K   K   L   L   Q   E   E   V   T   K   V   T   K   F   I   E   G   G   D    768

2350 GGT CAT TTA TTT GAA GAT GAA GAA ATT AAA AGA CTG CTT CAG GGA GAC ACA CCC GTG AGG 2409
 769 G   H   L   F   E   D   E   E   I   K   R   L   L   Q   G   D   T   P   V   R    788

2410 AAG TTG CAA GCC AAC AAA AAA AGT TCA AGG ATC TAG aagacgattaagggaaggtcgttctcagtgaa 2477
 789 K   L   Q   A   N   K   K   S   S   R   I   *                                    800

2478 aatccaaaaaccagaaaaaaatgtttatacaaccctaagtcaataacctgaccttagaaaattgtgagagccaagttgac 2557

2558 ttcaggaactgaaacatcagcacaaagaagcaatcatcaaataattctgaacacaaatttaatatttttttttctgaatg 2637

2638 agaaacatgagggaaattgtggagttagcctcctgtggtaaaggaattgaagaaaatataacaccttacaccctttttca 2717

2718 tcttgacattaaaagttctggctaactttggaatccattagagaaaaatccttgtcaccagattcattacaattcaaatc 2797

2798 gaagagttgtgaactgttatcccattgaaaagaccgagccttgtatgtatgttatggatacataaaatgcacgcaagcca 2877

2878 ttatctctccatgggaagctaagttataaaaataggtgcttggtgtacaaaactttttatgatcaaaaggctttgcacat 2957

2958 ttctatatgagtgggtttactggtaaattatgttatttttacaactaattttgtactctcagaatgtttgtcatatgct 3037

3038 tcttgcaatgcatatttttaatctcaaacgtttcaataaaaccatttttcagatataaagagaattacttcaaattgag 3117

3118 taattcagaaaaactcaagatttaagttaaaaagtggtttggacttgggaataggactttatacctctttctcgtgcc 3195
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FIG. 28D

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/12502

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/30 G01N33/53 G01N33/574 C12Q1/68 A61K39/395
G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K G01N C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 562 508 (HOECHST JAPAN LTD.) 29 September 1993 see tables see sequence listing ---	1-22
A	MOLECULAR BIOLOGY OF THE CELL, SUPPLEMENT, vol.4, 1993, BETHESDA MD, USA page 357A S. BAO ET AL. 'Identification and isolation of differentially expressed genes by palindromic PCR.' see abstract 2069 ---	3,7,9,17,19
A	WO,A,93 04198 (BRITISH TECHNOLOGY GROUP LTD.) 4 March 1993 see the whole document --- -/--	3,7,17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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1

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 1995	20 -03- 1995

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/12502

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 549 107 (SYNTEX (U.S.A.) INC.) 30 June 1993 see examples see figures see claims -----	3,7,17

1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 94/12502

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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WO-A-9304198	04-03-93	AU-A-	2379692	16-03-93
		CA-A-	2115829	04-03-93
		EP-A-	0613501	07-09-94
		GB-A-	2258867	24-02-93
		JP-T-	6510185	17-11-94
		NZ-A-	243922	26-10-94
EP-A-0549107	30-06-93	CA-A-	2080305	12-04-93